"A PROSPECTIVE, RANDOMIZED, OPEN LABEL, COMPARATIVE STUDY OF ATORVASTATIN ALONE AND ATORVASTATIN WITH LYCOPENE IN PATIENTS WITH HYPERLIPIDAEMIA ATTENDING TERTIARY CARE HOSPITAL"

Dissertation submitted to

THE TAMILNADU

DR. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment for the award of the degree of

DOCTOR OF MEDICINE

IN

PHARMACOLOGY

INSTITUTE OF PHARMACOLOGY
MADRAS MEDICAL COLLEGE
CHENNAI - 600 003

APRIL 2015

CERTIFICATE

This is to certify that the dissertation entitled, **"A PROSPECTIVE, RANDOMIZED, OPEN LABEL, COMPARATIVE STUDY OF ATORVASTATIN ALONE AND ATORVASTATIN WITH LYCOPENE IN PATIENTS WITH HYPERLIPIDAEMIA ATTENDING TERTIARY CARE HOSPITAL"** submitted by Dr. S. SUGANESHWARI, in partial fulfillment for the award of the degree of Doctor of Medicine in Pharmacology by The Tamilnadu Dr.M.G.R.Medical University, Chennai is a Bonafide record of the work done by her in the Institute of Pharmacology, Madras Medical College during the academic year 2012-2015.

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CERTIFICATE OF THE GUIDE

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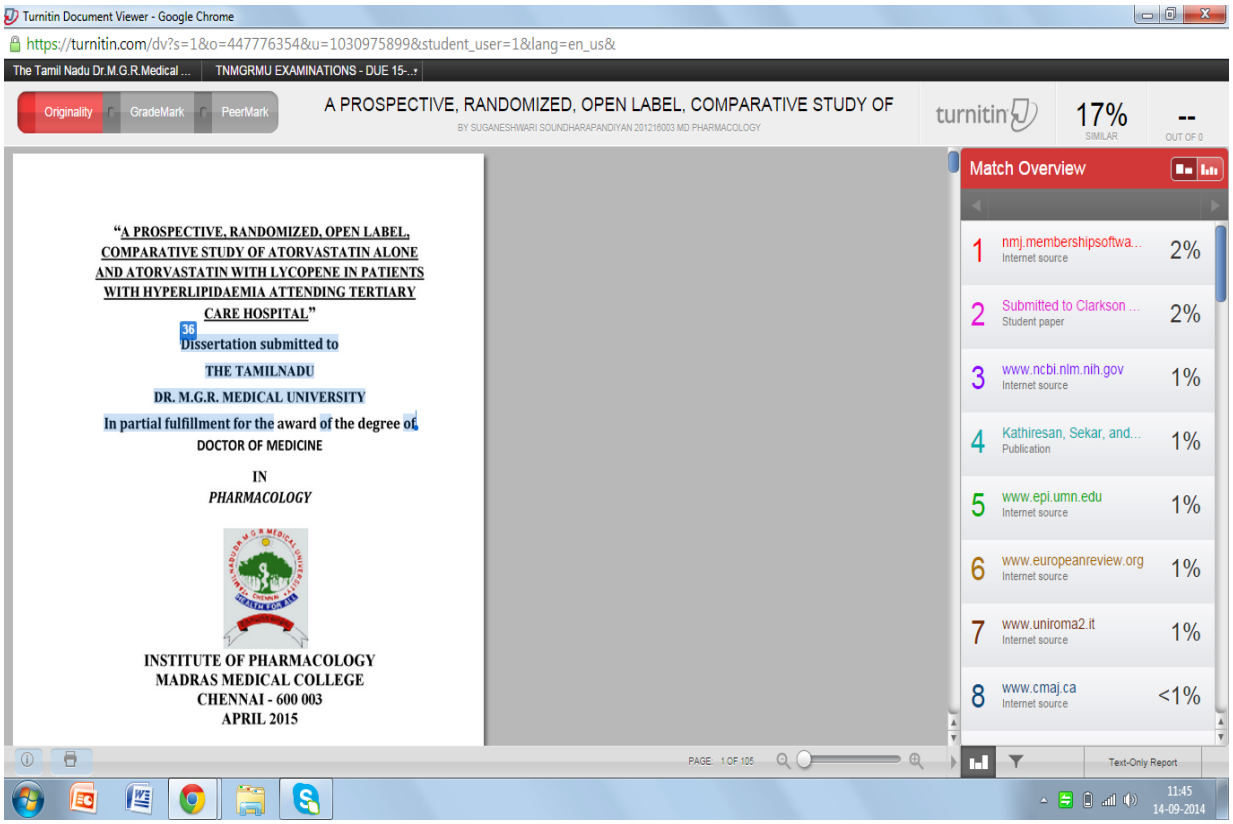
TURNITIN ANTI-PLAGIARISM SOFTWARE



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Dr. Suganeshwari S, Dr. Kalaiselvi B, Dr. Nandini R.

ABSTRACT

AIM:

1. To compare the efficacy and tolerability of Atorvastatin alone and Atorvastatin with Lycopene in the management of Hyperlipidaemia.

METHODOLOGY

This was an open label, comparative, randomized, prospective study. This study included 100 patients with Hyperlipidaemia, who were randomized into two groups of 50 each. One group received T.Atorvastatin 10mg/day and other group received T.Atorvastatin 10mg/day with T.Lycopene 15mg/day for 8 weeks. They received routine follow-up fortnightly for 8weeks. Lipid profile was assessed at baseline and at the end of the study.

RESULTS

The baseline characteristics were similar in both the study groups. On comparing the groups at the end of 8weeks there was a statistically significant reduction in Total cholesterol and LDL cholesterol levels. (TC - $p < 0.001$; LDL- $p < 0.004$). The hematological, hepatic and renal function test did not show any significant change when compared to baseline. Minimal adverse effects were observed in both the study groups.

CONCLUSION

1. From this study, we can conclude that Atorvastatin with Lycopene was effective in reducing the lipid levels.
2. The combination was well tolerated.

KEY WORDS

Atorvastatin, Lycopene.

INTRODUCTION

Cardiovascular diseases are one of the most common group of diseases causing increased pre-mature mortality and morbidity in both developing and developed countries. Atherosclerosis affecting the arterial vessel wall and the resulting thrombosis are the main pathogenic processes causing increased risk of cardiovascular diseases.

The main clinical entities of CVD are coronary artery disease, ischaemic stroke, congestive heart failure, hypertension, peripheral arterial disease, and congenital heart disease[1].

Various epidemiological factors have been attributed as risk factors for CHD (Coronary Heart Disease). Age, male individuals, hyperlipidemia, Type II diabetes mellitus, and smoking are some of the important risk factors for CHD. Hypercholesterolemia, is the term comprising elevated low-density lipoprotein (LDL) levels, and low high-density lipoprotein (HDL) levels and are unequivocally linked to increased risk for coronary heart disease and cerebrovascular disease. The LDL cholesterol levels are the primary target[2]. Thus, Hypercholesterolemia is a significant modifiable cardiovascular risk factor, and the risk is directly proportional to the degree of cholesterol elevation.

The most common cause of death is Coronary Heart Disease worldwide. In India, approximately 46.9 million patients presented with cardiovascular disease in 2010. An estimated death of 2.3 million people was recorded during 2008. A drastic increase of 23.3 million deaths is expected in the year 2030 due to cardiovascular disease[3].

According to World health organization, CHD is considered as our modern EPIDEMIC i.e., a disease that affects population not an unavoidable attribute of aging.

In India, the burden of Ischaemic Heart Disease is increasing every year, because of increased exposure to risk factors like unhealthy diet, lifestyle modification, smoking, obesity etc. By the year 2015, it may predominate as the single most important cause of death[4].

The stress induced lifestyle is most common in our day to day activities which also more prone for causing increased oxidative stress. The release of reactive oxygen species is increased due to oxidative stress which contributes as the aetiology of the several chronic diseases[5].

The endogenous antioxidants are depleted to overcome the increased oxidative stress. Thus the importance of the antioxidant rich vitamins like Vitamin A, E, C in the prevention of these chronic heart diseases is being stressed nowadays. Nutrients rich in antioxidants are modestly attributed to decrease the tissue damage which prevents the atherosclerotic process[6].

Apart from vitamins, certain antioxidants are being present in the plants which are most commonly used. Among natural antioxidant, lycopene is an extract obtained from tomatoes which is one of the most potent and most effective antioxidant. It helps in preventing the oxidation of lipids with its single-oxygen quenching ability two times more potent than β-carotene and 10 times than α-tocopherol[7].

It has a role in altering the metabolism and oxidation of lipids which predispose to the formation of atherosclerosis.

Lycopene shows 37% decrease in synthesis of cholesterol in J-774A.1 macrophage cell line, and it also augment the activity of macrophage LDL receptors. Macrophage enrichment with lycopene results in the suppression of cellular cholesterol

synthesis by inhibiting the enzyme, HMG-CoA reductase. This effect leads to increased clearance of LDL from the plasma and due to this effect lycopene is being recognized as hypolipidemic agent[8].

Hence, the present study was planned to assess the efficacy of Lycopene in addition to atorvastatin in reducing total cholesterol and LDL-C and also to compare with that of atorvastatin when used alone.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Cardiovascular diseases (CVD) are one of the leading causes of morbidity and mortality in the world. Increase in cholesterol level is a predominant risk factor for atherosclerosis and associated coronary and cerebro-vascular diseases.

The presence of high level of cholesterol in the blood is termed as Hypercholesterolemia. Higher the level of low density lipoprotein cholesterol, higher risk for atherogenesis. The increased level of LDL occurs due to inadequate removal of cholesterol from macrophages by HDL cholesterol. These increased levels of LDL-C are oxidized by reactive oxygen species. The oxidised LDL-C are further scavenged by the macrophages which results in foam cell formation and accumulation of numerous adhesion factors, monocytes and neutrophils on the vascular lumen. The resultant atherosclerosis is the predisposing factor for various cardiovascular diseases which affects the quality of life.

So, to lead a healthy life a balance in cholesterol homeostasis is important which can be achieved by a gene network involved in cholesterol synthesis, absorption, metabolism and elimination.

LIPID METABOLISM

Lipid metabolism is a process that involves synthesis and degradation of lipids or cholesterol. Lipids are heterogeneous group of compounds, including fats, oils, steroids, and waxes, which are related mainly by their physical properties.

They are water insoluble but it is carried within the protein particles called lipoproteins in the blood plasma. They are stored as a source of energy production.

Types of lipids:

1. Simple
2. Complex / Compound
3. Other (Neutral) lipids

1. Simple lipids

These are esters of fatty acids with various alcohols. They are,

i. Lauric acid and palmitic acids are saturated fatty acids which have sizeable effect on increasing blood cholesterol level. Most hydrogenated vegetable oils like “Dalda” have saturated fatty acids.

ii. Oleic acid and palmitoleic acids are monounsaturated fatty acids having one double bond in their structure. They are mostly present in olives and nuts. They are useful in decreasing LDL and VLDL cholesterol levels when compared to saturated fatty acids.

iii. Linolenic acid and arachidonic acids are polyunsaturated fatty acids. The structure comprises of 2 or more double bonds. They have a lipid lowering effect. They are abundant in safflower oil, corn oil, and fish oil.

iv. Elaidic acid is unsaturated fatty acid which is generated during hydrogenation of polyunsaturated fatty acids and monounsaturated fatty acids. These are present in some commercial vegetable oils. They are harmful because of their increasing LDL cholesterol and lowering HDL cholesterol levels.

2. Complex / Compound lipids

They are fatty acids esters containing groups like phosphoric acid residue, spingosine in their structure. e.g. phospholipids and sulfolipids.

3. Other lipids (Neutral lipids)

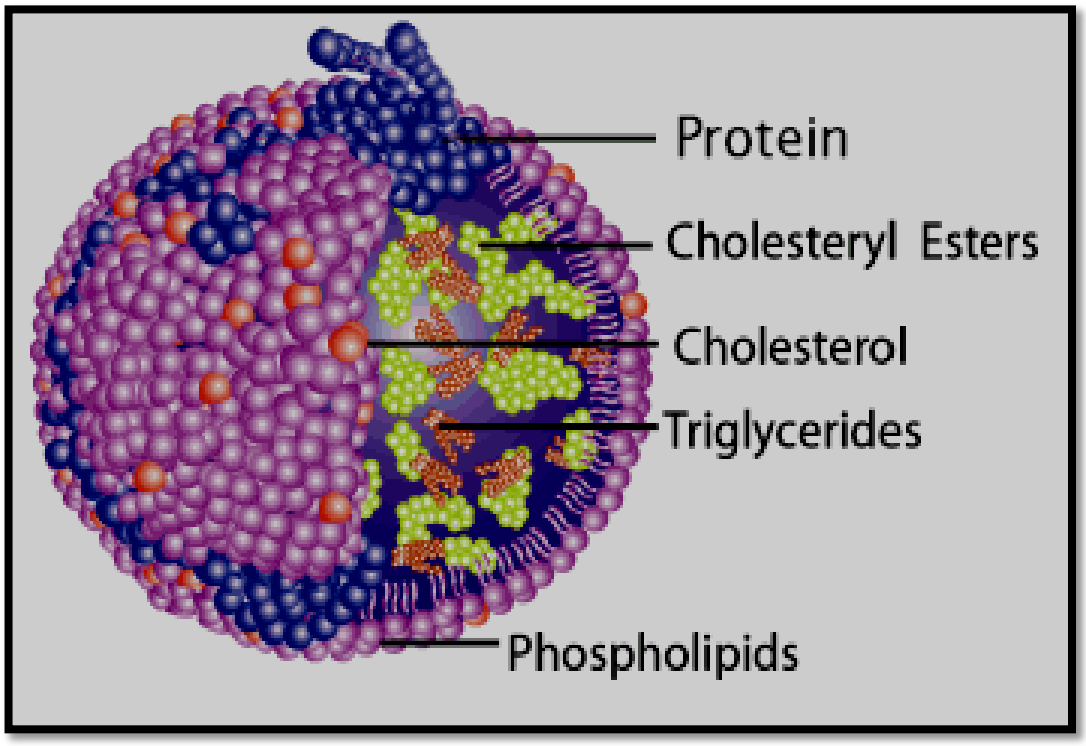
The non-polar lipids include Cholesterol (CH), Triglycerides (TGs), and Cholesterol esters (CE). Triglycerides are glycerol with its structure containing all three hydroxyl groups linked to fatty acids. Cholesterol is said to be sterol chemically but not a fat. They are transported as solubilised form in the blood stream with the help of lipoproteins[9].

LIPOPROTEIN METABOLISM

The biochemical assembly of lipids and proteins are called lipoproteins. These are important cellular constituents in the cell membrane and mitochondria. Lipoproteins are essential for the transport of cholesterol and triglycerides. Lipoproteins play an important role in the dietary cholesterol absorption and transport of lipids from the intestine as chylomicrons and from the liver as very low density lipoproteins (VLDL- C). These are transported to most of the tissues for oxidation and for storage in adipose tissue[10].

The structure of lipoprotein is made up of a hydrophobic core containing triglycerides and cholesteryl esters which are surrounded by a hydrophilic lipid containing phospholipids and unesterified cholesterol.

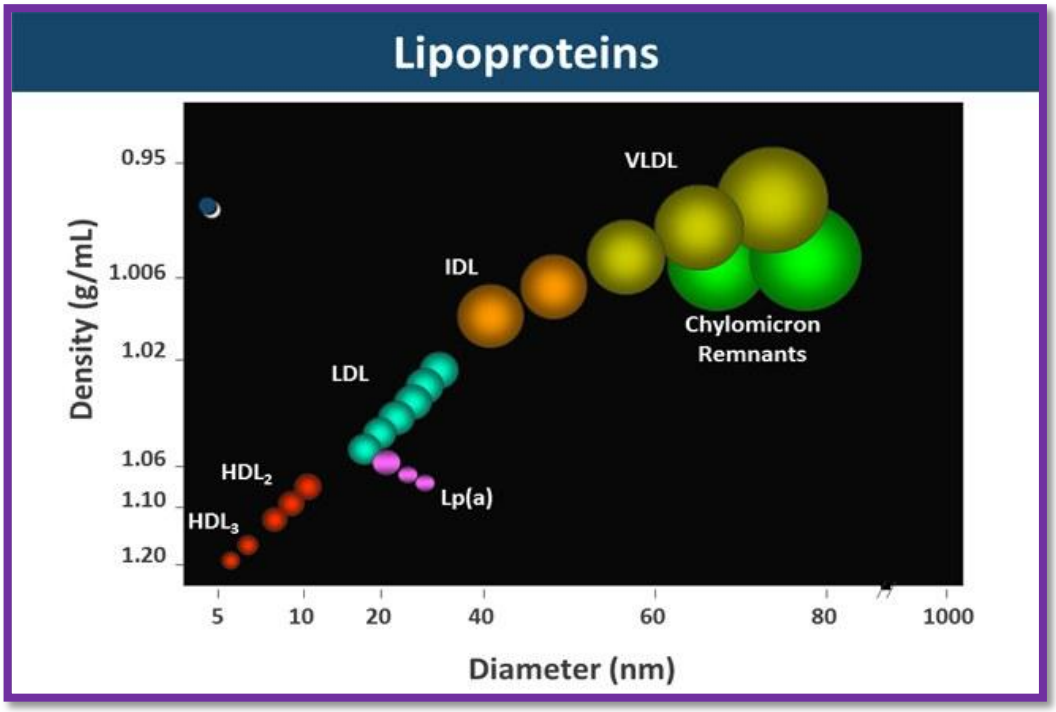
Structure of Lipoprotein



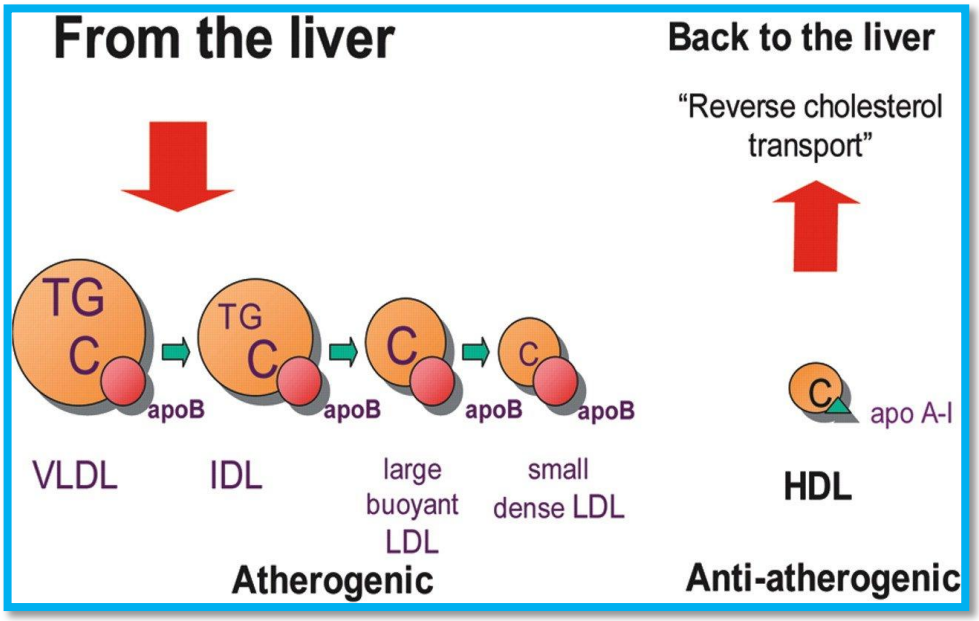
Based on their relative density and diameter, the lipoproteins are classified into five types. It is described from larger and less dense to smaller and denser. They are considered as larger and less dense when the fat to protein ratio is increased.

1. The lipoprotein with greater diameter and lower density is known as **Chylomicrons,** which are derived from absorption of triacylglycerol and other lipids from the intestine.
2. **Very low density lipoproteins** (VLDL, or pre-ß-lipoproteins) with lower diameter and higher density than chylomicrons, which are derived from the liver for the transport of newly synthesized triacylglycerol.
3. **Intermediate density lipoprotein (IDL)** is a lipoprotein with lower diameter and higher density than VLDL. They are usually not measurable in blood during fasting.

Major classes of Lipoprotein particles



4. **Low-density lipoproteins** (LDL, or ß lipoproteins), represents a final stage in the catabolism of VLDL. They are termed as "bad cholesterol" due to increased progression of atherosclerosis.
5. **High-density lipoproteins** (HDL, or α-lipoproteins), with highest density and lower diameter involved in reverse cholesterol transport carrying fat molecules from the tissue back to the liver. They are termed as "good cholesterol" due to their protective effect from atherogenesis.



APOLIPOPROTEINS

Apolipoproteins are proteins which bind with lipids to form lipoproteins. The main functions of apolipoproteins are

- They form the structure of lipoprotein eg, apo B.

- They act as enzyme cofactors or enzyme inhibitors.

  Eg,

  C-II and A-I are enzyme cofactors.

  - Lipoprotein lipase – C II
  - Lecithin:cholesterol acyltransferase – A I

  A-II and C-III are enzyme inhibitors.

  - Lipoprotein lipase - Apo A-II and C-III

- They act as ligands

  Eg,

  - LDL receptor - Apo B-100 and apo E
  - LDL receptor related protein - Apo E
  - HDL receptor - Apo A-I

EXOGENOUS PATHWAY OF LIPIDS

Fat-soluble vitamins, dietary cholesterol and fatty acids are absorbed in the proximal part of the small intestine. Inside the intestinal lumen, lipases hydrolyse the dietary triglycerides and emulsify with bile acids to form micelles.

In the enterocyte, by the addition of a free fatty acid the cholesterol esterification occur which results in the formation of cholesteryl esters. Incorporation of triglycerides with fatty acids containing more than 12 carbons atoms are packed with apo-B48, cholesteryl esters, retinyl esters, phospholipids, and cholesterol resulting in the formation of chylomicrons.

The newly secreted chylomicrons are called nascent chylomicrons which are absorbed into the intestinal lymph and carried directly through the thoracic duct to

the blood stream. They are transported to the peripheral tissues before entering the liver.

In heart, skeletal muscle and adipose tissue these nascent chylomicrons are attached to the lipoprotein lipase anchored by a protein called phosphatidylinositol-anchored protein, GPIHBP1. These reactions occur mainly on the endothelial surface of the capillaries. They are hydrolysed by the lipoprotein lipase and the free fatty acids are released. HDL transfers the apo C-II to the chylomicron that acts as a cofactor for lipoprotein lipase.

The released free fatty acids are taken up by heart and skeletal muscles which are oxidized to generate energy. They can also be re-esterified and stored as triglyceride. Some of the free fatty acids released will enter into the hepatocytes by binding with the plasma protein like albumin.

Due to hydrolysation of its hydrophobic core the resultant chylomicrons progressively decrease in size. The hydrophilic lipids like cholesterol, phospholipids and the protein moiety apolipoproteins on the particle surface are transferred to HDL. These result in the formation of a chylomicron remnant which is about half the diameter of nascent chylomicron.

The chylomicron remnants are mainly made up of cholesterol and cholesteryl esters. These remnants are rapidly taken up by the liver from the circulation where apo-E act as a ligand.

ENDOGENOUS PATHWAY OF LIPIDS- HEPATIC LIPIDS

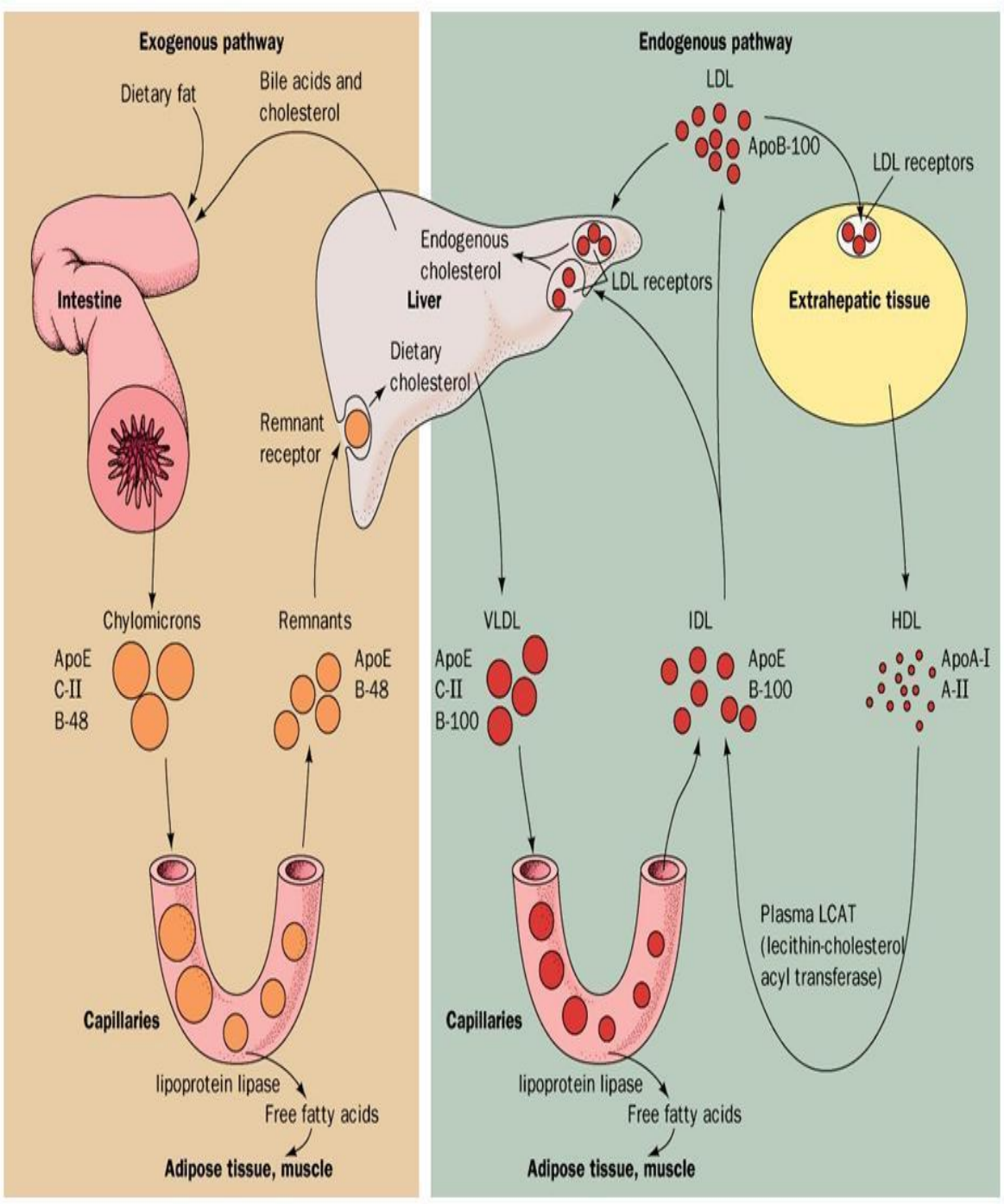
The endogenous transport of cholesterol mainly involves

- The liver which secretes apo-B lipoproteins and
- The peripheral tissues where the triglycerides particles are metabolized.

The VLDL particles resemble chylomicrons in protein composition, where the apo-B48 is replaced by apoB-100. They have the higher ratio of cholesterol and triglycerides.

The triglycerides present in the very low density lipoprotein are derived mainly from the esterification of long-chain fatty acids in the liver. The process of combining the hepatic triglycerides with the other major components of the nascent VLDL particle like apoB-100, phospholipids and cholesteryl esters are acquired by the action of the enzyme protein called microsomal triglyceride transfer protein (MTP).

Diagram shows both exogenous and endogenous pathway



In the plasma, HDL transfers the apo-E and the C series of apolipoproteins to the VLDL particle. In the heart, skeletal muscle and adipose tissue the triglycerides of the VLDL particle are hydrolysed by the lipoprotein lipase enzyme similar to the process occurring in to the chylomicron. This results in the formation of VLDL

remnants which dissociated from the lipoprotein lipase. Then these remnants are called as IDL (intermediate density lipoprotein).

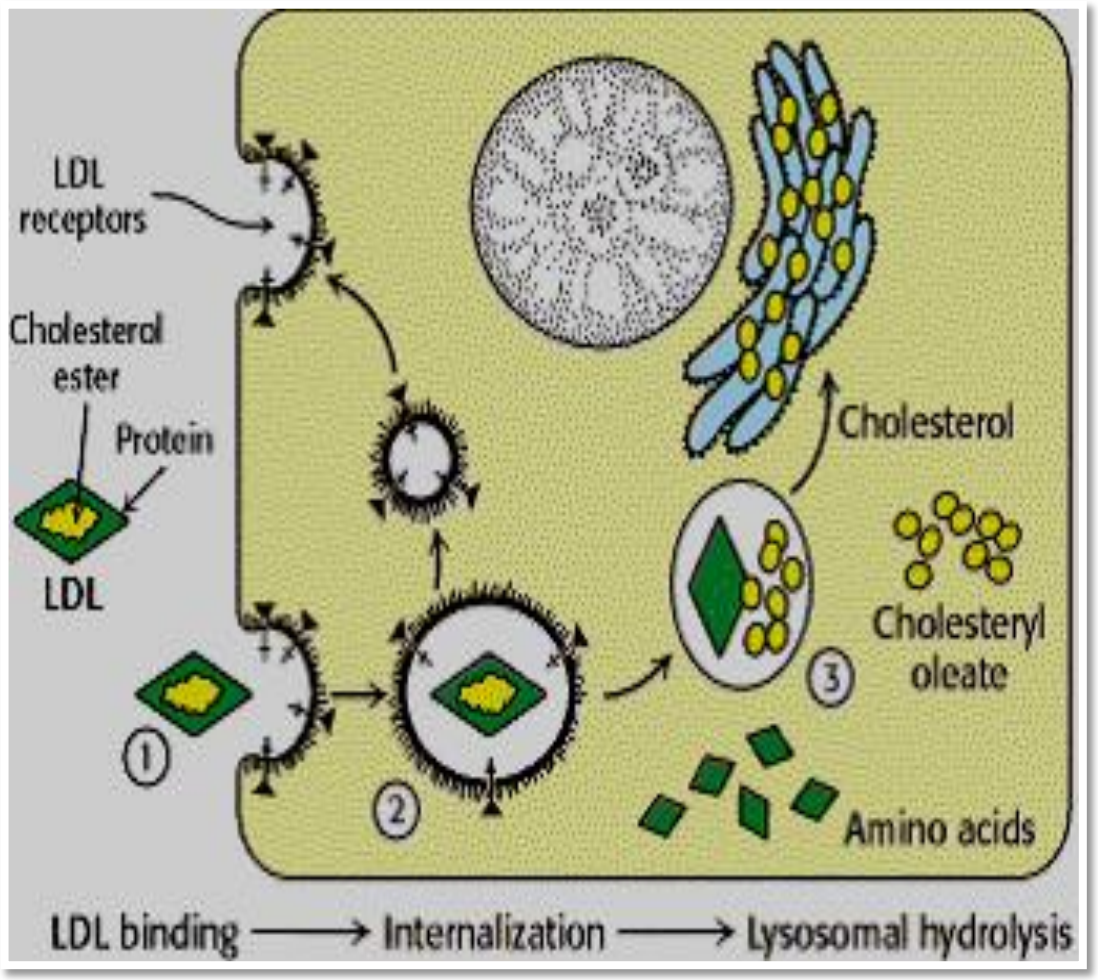
IDL contain almost same amounts of triglyceride and cholesterol. 40-60% of IDL particle are removed by the liver through endocytosis by binding to apo-E and apoB-100. The remaining IDL is remodeled by hepatic lipase enzyme to form LDL.

In this process most of the triglycerides are hydrolysed and results in the formation of LDL which carries apoB-100. In most of the individuals the concentration of plasma cholesterol is equivalent to the amount of cholesterol present in the LDL particle. In the liver, circulating LDL cholesterol of about 70% is cleared by LDL receptor-mediated endocytosis.

Receptor-mediated endocytosis of low density lipoprotein[11,12]**:**

1. In the non-hepatic cells, apo B-100 over the surface of a LDL particle binds to a specific receptor protein called clathrin. The receptors for LDL are localized in a specialized region called coated pits containing the receptor protein.
2. The LDL- receptor complex is engulfed by a process called endocytosis resulting in the formation of an endocytic vesicle.
3. The endocytic vesicle later fuse with lysosomes which carries a wide array of degradative enzymes. The protein and cholesteryl ester of low density lipoprotein are hydrolysed by lysosomal enzyme, lipase. The free amino acids are released from the protein component. The cholesteryl esters present in the LDL particle are hydrolyzed by a lysosomal acid lipase. The LDL receptor itself usually returns unmodified to the plasma membrane. The turn- around time for a receptor is about 10 minutes. In its lifetime of about a day, it may bring many LDL particles into the cell.

Receptor mediated endocytosis



4. The un-esterified cholesterol can be used for membrane biosynthesis and also it can be re-esterified and stored inside the cell. In fact, free cholesterol activates acyl CoA: cholesterol acyltransferase (ACAT), the enzyme catalyzing this reaction.
5. Oleate and palmitoleate are the monounsaturated fatty acids present in the re-esterified cholesterol while the cholesterol esters in LDL contain linoleate, a polyunsaturated fatty acid. High concentrations of unesterified cholesterol disrupt the integrity of cell membranes.

6. The synthesis of LDL receptor is itself subjected to feedback regulation. When cholesterol is abundant inside the cell, synthesis of new LDL receptors does not occurs and thus the uptake of further cholesterol from plasma is inhibited.

LDL-C ROLE IN LIPID METABOLISM

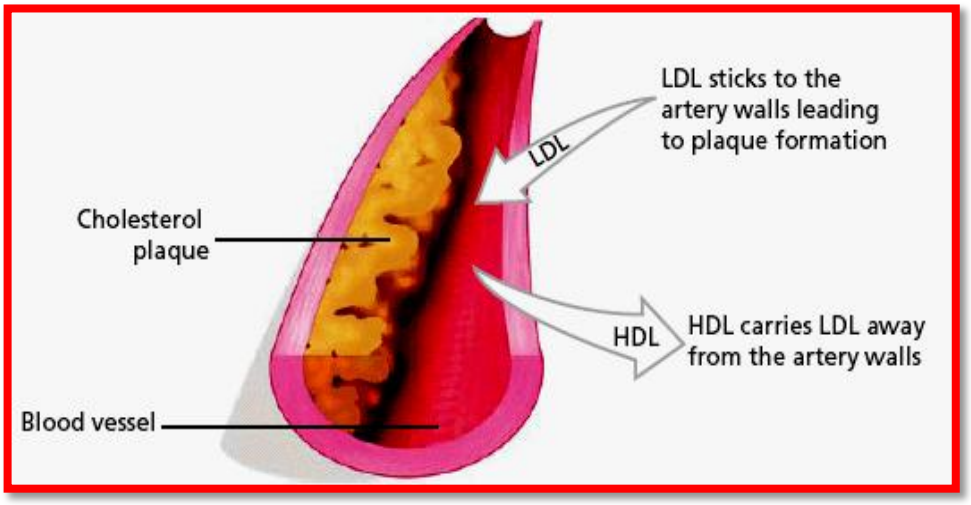
The metabolism of cholesterol is regulated precisely to prevent the formation of atherosclerosis. Since the major carrier of cholesterol in the blood is low density lipoprotein, it plays a major role in the formation of atherosclerosis. The main role of LDL-C is to transport the cholesterol to peripheral tissues.

Control of cholesterol synthesis in liver

In the liver, low density lipoproteins controls the synthesis of cholesterol by reducing the effect of 3- hydroxy-3-methylglutaryl CoA reductase enzyme.

Control of cholesterol synthesis in non-hepatic cells

The Low density lipoproteins are the primary source of cholesterol in the non-hepatic cells. In most cases of familial hypercholesterolemia, the major molecular defect is a deficiency or absence in LDL receptor. Thus the increased level of LDL-C is deposited in various tissues. Atherosclerotic lesion contains mainly the oxidized lipids derived from the LDL-C.

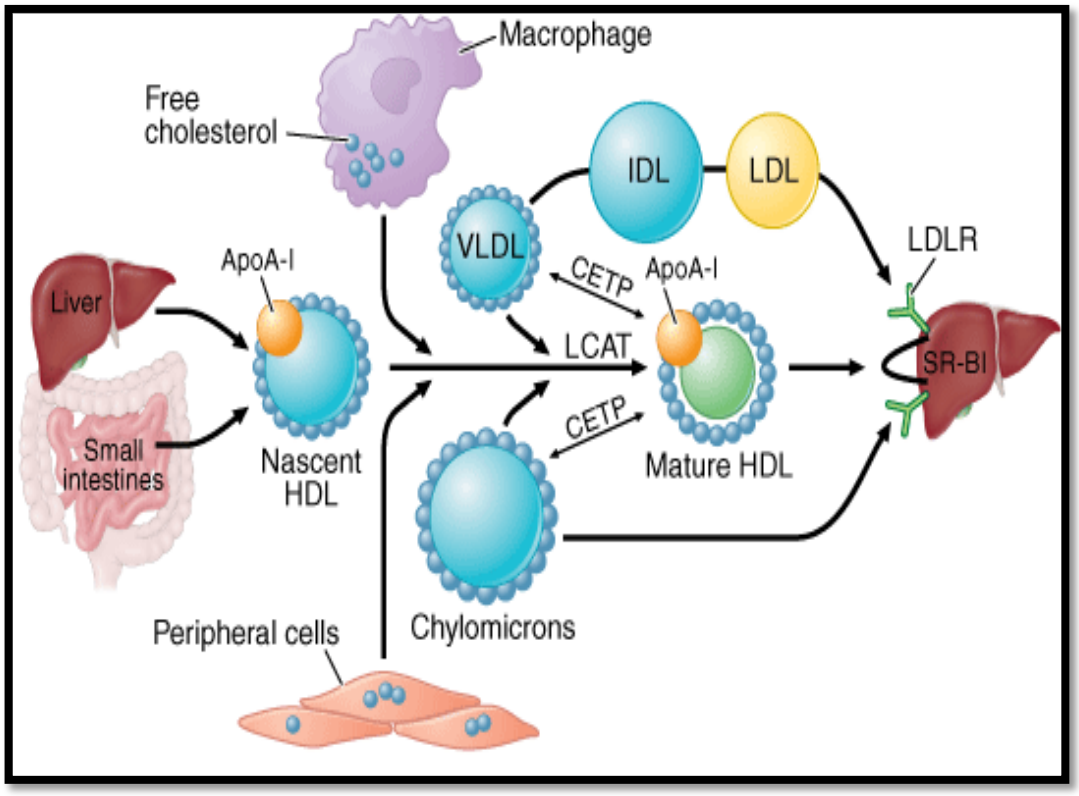


ROLE OF HDL

Apolipoproteins of high density lipoproteins are secreted by the intestine and liver. High density lipoprotein derives cholesterol from the surface monolayers of chylomicrons and VLDL. It also acquires cholesterol from the peripheral tissues, which protects the cells to maintain cholesterol homeostasis.

From the cell membrane the free cholesterol is mainly transported by a transporter called ABCA1, which is acquired by a small particle termed prebeta-1 HDL. The mature HDL particle is formed by the esterification of the resultant nascent HDL by lecithin:cholesterol acyl tranferase (LCAT).

Reverse cholesterol transport



The free cholesterol expelled from the macrophages by means by an ABCA1 transporter, which results in the formation of the mature HDL particles. The cholesteryl esters from the mature HDL particle are transferred to chylomicrons remnants, VLDL,

IDL and LDL, by means of a transfer protein called Cholesteryl ester transfer protein (CETP).

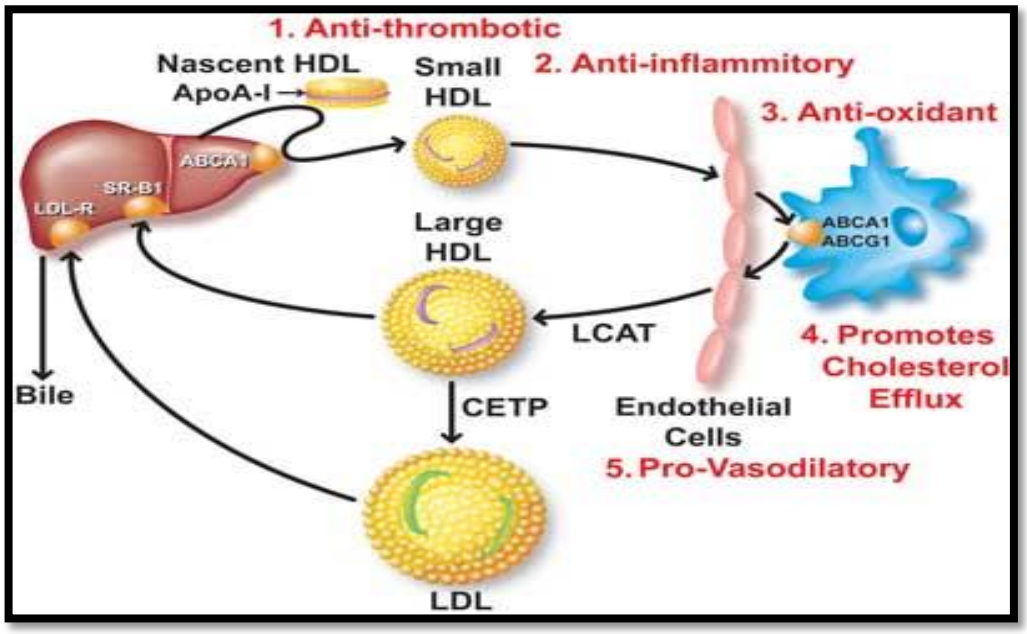
The transferred cholesteryl ester from the HDL particle is finally taken up by the liver via two processes

- Endocytosis.
- Docking receptor or scavenger receptor, SR-B1[13].

The process involved in the HDL metabolism is called reverse cholesterol transport mechanism.

PROTECTIVE ROLE OF HDL

Protective role of HDL-C



1. Has an anti-inflammatory property.
2. Act as an anti-oxidant by improving endothelial function through stimulating the endothelial nitric oxide production.
3. Inhibition of cell adhesion molecules expression such as vascular cell adhesion molecule -1 (VCAM-1), E-Selectin, intercellular adhesion molecule-1, and also the accumulation of inflammatory infiltrates in the vessel wall.

HYPERLIPIDEMIA

DEFINITION OF HYPERLIPIDEMIA

Dyslipidemia refers to the alteration of one or many of the lipoproteins which may be an elevation of triglycerides or low density lipoproteins cholesterol, or decrease in high-density lipoprotein cholesterol, while lipid elevation alone is termed as 'Hyperlipidemia'[14].

Various terms,

- Hyperlipidemia – increase in lipid levels,
- Hypercholesterolemia – increase in cholesterol levels,
- Hyperlipoproteinemia – increase in lipoprotein levels.

HYPERLIPOPROTEINEMIA- CAUSES[15]

1. Environmental factors
2. Genetic factors
3. Secondary causes

1. Environmental factors

Dietary factors and obesity.

2. Genetic factors

Occur due to single gene or multiple gene defects

CLASSIFICATION OF HYPERLIPOPROTEINEMIA - FREDRICKSON-LEVY-LEES [16]

Familial Hyperchylomicronaemia – Type I

- This type usually presents as massive fasting hyperchylomicronemia. There is rise in serum triglyceride levels even after normal fat intake in diet.
- Lipoprotein lipase or normal apolipoprotein C-II (rare) deficiency is the cause.

- Type I usually does not increase risk of coronary heart disease.
- No drug therapy is effective but low fat diet should be followed.

Familial Hyperchylomicronaemia – Type II A

- LDL-C degradation is halted causing rise in LDL-C with normal VLDL-C levels. Serum cholesterol level is raised, triglyceride levels are normal.
- Defects in the synthesis or processing of LDL-C receptors are the causes.
- Risk of Ischaemic heart disease is highly increased.
- Proper diet can be an effective treatment.
- In heterozygotes: Drug therapy like cholestyramine, statin or niacin.

Familial combined or mixed Hyperlipidemia – Type II B

- Similar to IIA, but here VLDL-C is increased, resulting in elevated serum triglycerides and cholesterol levels.
- Overproduction of VLDL-C in the liver is the cause and relatively common.
- Treatment: Diet modulation and drugs. Treatment is similar to Type IIA treatment.

Familial dysbetalipoproteinemia – Type III

- Mutation of apo E cause increased production or under utilization of IDL cholesterol which results in increased serum levels. These also cause increased triglycerides and cholesterol levels.
- Causes xanthomas and acceleration of vascular disease in middle aged patients.
- Treatment: Diet modulation and drugs niacin and fenofibrate or statin.

Familial Hypertriglyceridaemia – Type IV

- Increased VLDL-C levels, normal or decreased LDL-C levels, normal to increased cholesterol, highly increased circulating triglyceride levels are the features.

- The increased VLDL cholesterol level is due to overproduction and/or impaired removal of serum VLDL-C triglycerides.
- It is relative common disease with few clinical manifestations apart from accelerated ischaemic heart disease like obesity, diabetes and hyperuricaemia.
- Treated with diet modulation and drug therapy like niacin and/or fenofibrate.

Familial mixed Hypertriglyceridemia – Type V

- Raised VLDL-C and chylomicron levels, normal or reduced LDL-C causing increased cholesterol and greatly increased triglyceride levels.
- The increased levels are due to either over production or reduced clearance of VLDL-C and chylomicrons due to a genetic defect.
- Affected patients are adults with obesity and diabetes.
- Treatment: Diet, drugs includes niacin, and/or fenofibrate.

SECONDARY CAUSES [17]

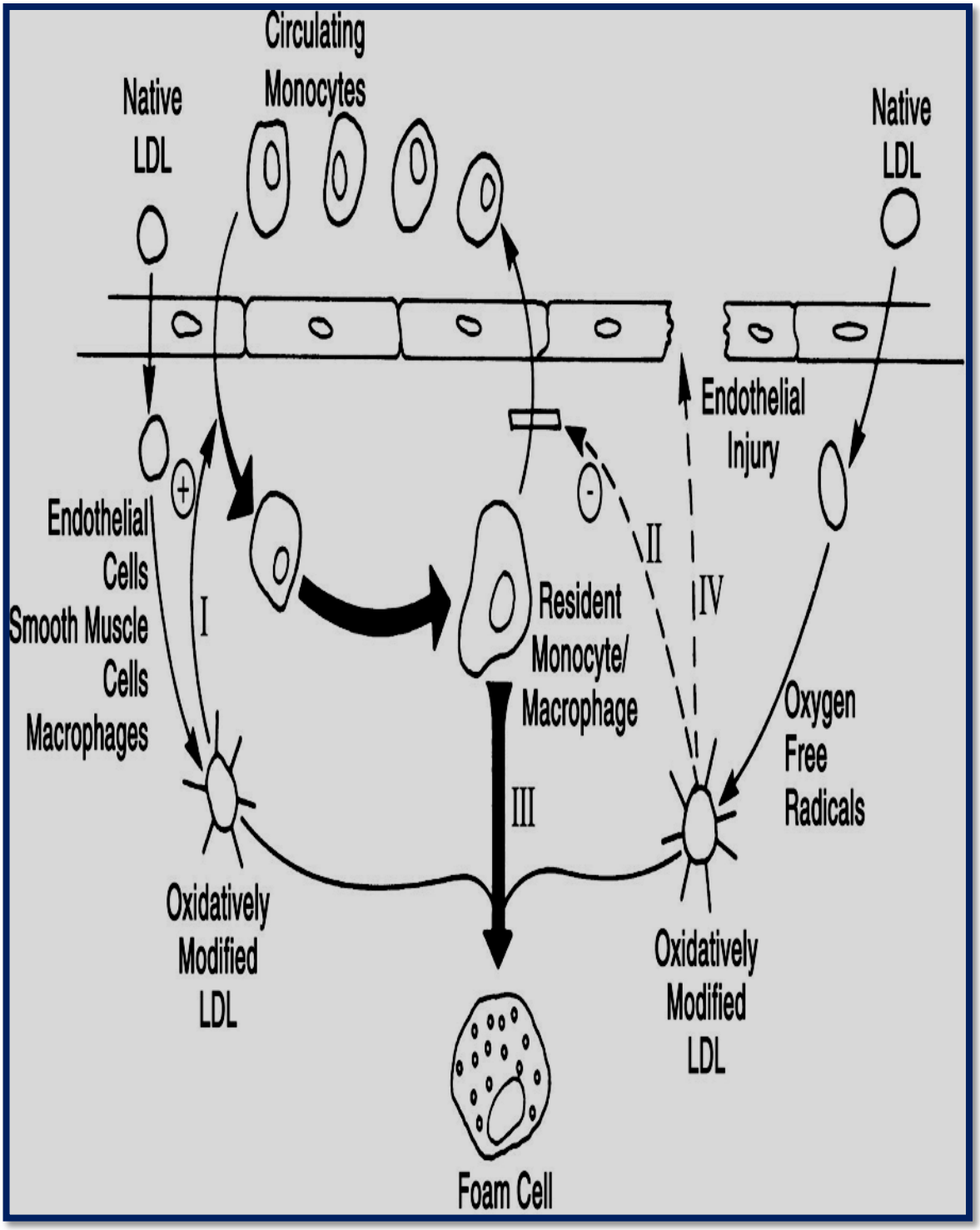
- Diabetes mellitus
- Hypothyroidism
- Lipodystrophy
- Stress
- Alcohol excess
- Anti-hypertensive drugs, diuretics
- Glucocorticoid treatment
- Nephritic syndrome
- Acute intermittent porphyria
- Cholestasis
- Chronic renal failure
- Glycogen storage disease
- Pregnancy
- Sepsis
- Acute hepatitis
- Protease inhibitor therapy
- Obstructive liver disease
- Anorexia nervosa

HYPERLIPIDEMIA - RISK FACTOR FOR ATHEROSCLEROSIS.

The mechanism by which hyperlipidemia contributed to atherogenesis includes:

1. Accumulation of lipoproteins within the tunica intima of arterial wall mainly at the site of increased permeability.
2. An increased cholesterol level directly impairs the function of endothelium by increased production of oxygen free radicals. The impaired endothelium will cause decreased release of endothelial relaxing factor nitric oxide.
3. Low density lipoproteins are oxidized by the generated free radicals by the macrophages and endothelial cells results in formation of the oxidized LDL-cholesterol.
4. The resultant oxidized LDL cholesterol is readily ingested by the macrophages through scavenger receptor resulting in the formation of foam cells.

Role of Oxidized LDL-C in atherosclerosis



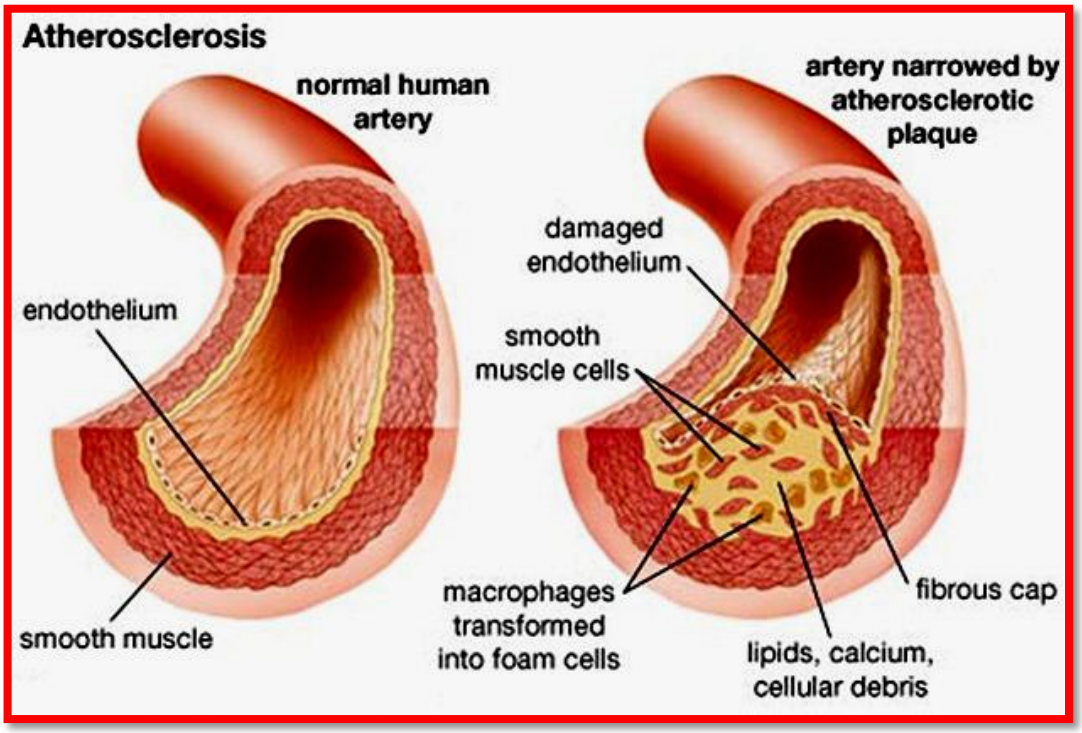
ATHEROSCLEROSIS

Atherosclerosis mainly involves the intima of the large and medium sized muscular arteries and is composed of fibrofatty cap (smooth muscle cells, lymphocyte, foam cell, collagen, macrophage, neovascularisation, proteoglycans, elastin) and necrotic center (foam cells, cell debris, calcium, cholesterol crystal)[18].

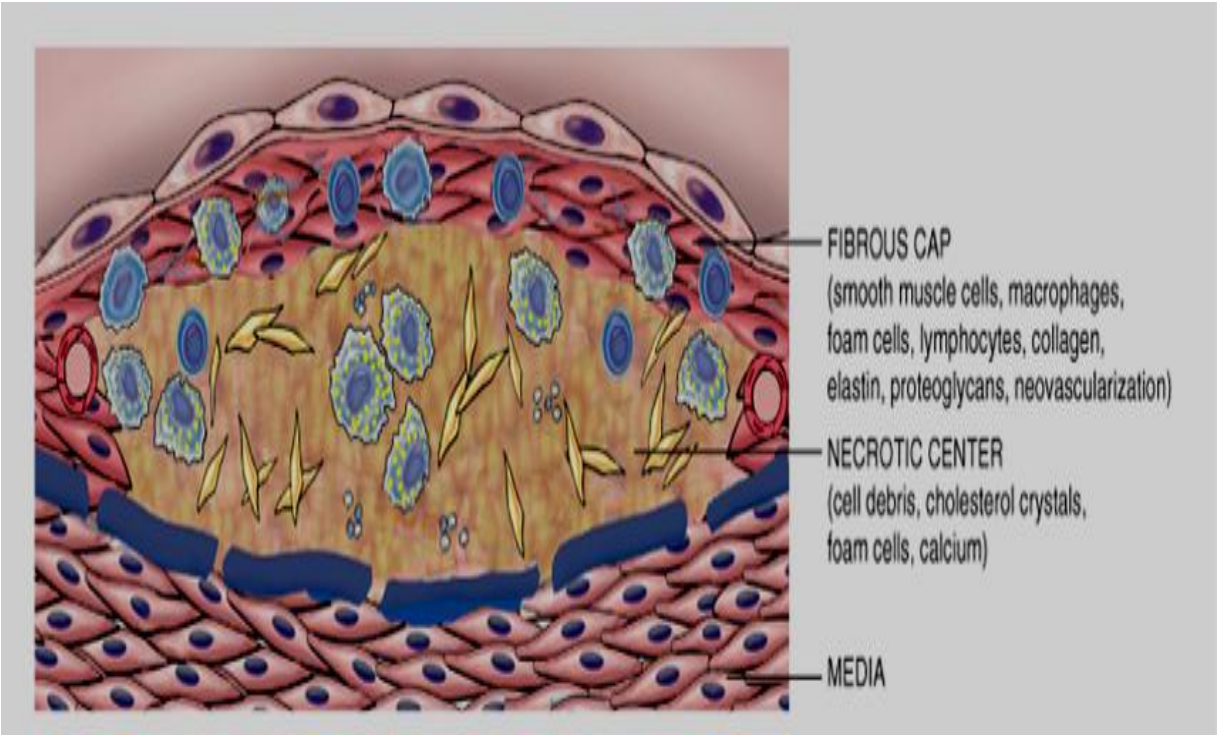
The term atheroma is derived from the greek word “athera” means lump of gruel. It is a chronic inflammatory response resulting from endothelial injury to the arterial wall causing invasion of leukocytes and formation of oxidized lipoproteins. It is commonly referred to as “hardening” or “furring” of arteries.

Atherosclerosis and its relevant vascular events involving coronary artery causing cardiovascular disease (CVD) and cerebral artery causing stroke and periphery artery causing peripheral arterial disease (PAD) have become one of the leading cause of disability and mortality[19].

Atherosclerosis



Pathogenesis of atherosclerosis



Pathogenesis of atherosclerosis[20]

Chronic injury to endothelial wall

Increased permeability, adhesion of leukocytes, and thrombosis

Lipoprotein accumulation (LDL and its oxidized forms) in the vessel wall

Adhesion of monocytes and migration into intima of vessel

Formation of macrophages and foam cells

Release of platelet adhesion factor from activated platelets, macrophages

Recruitment and proliferation of smooth muscle cell

Lipid accumulation both extracellular and intracellular (macrophages and SMCs)

RISK FACTOR FOR ATHEROSCLEROSIS

Major Non Modifiable	**Lesser, Uncertain**
Elderly	Obesity
Male sex	Physical Inactivity
Family history	High Carbohydrate diet
Genetic abnormalities	Stress (Type A personality)
	Post menopausal
	Estrogen deficiency
	Lipoprotein [LP(a)]
	Hardened unsaturated fat intake
Potentially controllable	
Hyperlipidemia	
Hypertension	Chlamydia pneumonia
Cigarette smoking	
Diabetes	
C-reactive protein	

Hyperlipidemia is one of the potentially controllable risk factors in causing atherosclerosis.

TREATMENT GUIDELINES FOR OPTIMAL PLASMA LIPID LEVELS:[21]

Lipid Levels classification:

Total cholesterol:

Desirable	less than 200 mg/dl
Borderline high	200-239 mg/dl
High	more than equal to 240 mg/dl

HDL cholesterol:

Low	less than 40 mg/dl (50 in women)
High	More than 60 mg/dl

LDL cholesterol:

Optimal for very high risk	Less than 70 mg/dl
Optimal	Less than100 mg/dl
Near optimal	100-129 mg/dl
Borderline high	130-159 mg/dl
High	160-189 mg/dl
Very High	≥190 mg/dl

Triglycerides

Normal	Less than 150 mg/dl
Borderline high	150-199 mg/dl
High	200-499 mg/dl
Very High	≥ 500 mg/dl

MANAGEMENT OF HYPERLIPIDEMIA

The treatment includes:

1. Modification of diet
2. Physical exercise
3. Avoidance of associated risk factors.
4. Medical management of Hyperlipidemia

1. MODIFICATION OF DIET

The primary treatment of hyperlipidemia is dietary modification. Drugs are added later to augment treatment. The "heart-healthy" diet is a food which contains low saturated fat. These provide a sufficient energy for the growth and maintain an adequate weight of an individual.

The specific dietary interventions are

(a) **Decreased intake of saturated fat**:

Decreased intake of saturated fat has the greatest impact in reducing LDL cholesterol levels in the blood. The foods containing saturated fats are hydrogenated peanut butters, partially hydrogenated oils and fats, commercial fried food, commercial bakery products and animal products containing fat.

(b) **Low intake of trans-fatty acids**:

Foods rich in trans-fatty acids are considered to increase the level low density lipoprotein cholesterol and it also decreases HDL cholesterol levels.

(c) **Decreased intake of dietary cholesterol**:

The intake of dietary cholesterol varies from individual to individual. The decreased intake of cholesterol diet helps in reduction of LDL-C levels. The hyperglycemic patients are more sensitive to increased dietary cholesterol intake, which are rich in animal fat.

(d) **Appropriate balance of fatty acid composition in diet**:

More consumption of monounsaturated and polyunsaturated fatty acids are better substitutes for saturated fats in lowering the LDL cholesterol levels.

(e) **Encourage omega-3-fatty acid intake**:

Omega-3-fatty acid consumption results in reduction TG levels and it also have cardio protective effects. Thus AHA recommends at least 1 fatty fish meal or other source of omega 3-fatty acids per week.

(f) **Increase dietary fiber intake**:

Soluble fiber intake will reduce the LDL cholesterol levels. Fruits, vegetables, cereals, oats, whole grains, and legumes are good sources of soluble fiber.

(g) **Encourage antioxidant food sources**:

There is valuable evidence regarding various antioxidants which plays a significant role in decreasing the incidence of coronary heart disease. Various antioxidants like carotenoids (Lycopene) and vitamins C and E have been attributed with lower cardiovascular risk. So, the recommended intake of foods rich in antioxidants such as tomatoes, citrus fruits, papaya, whole grains, melons, berries and dark orange/yellow or leafy green vegetables are helpful in reducing various cardiovascular events rather than other food supplements.

(h) **Reduce serum homocysteine levels**:

Coronary heart disease is also associated with high blood levels of homocysteine. Intake of folate, vitamins B_6, B_{12} and total fat restriction keep a check on homocysteine levels.

(i) **Avoid Plant sterols**:

Plant-sterol containing foods are associated with reducing low density lipoprotein cholesterol. But this is a double edged sword as it reduces the absorption of fat-soluble vitamins. Therefore, American Heart Association recommends these foods, such as "cholesterol-lowering" margarine-type spreads and salad dressings, mainly for adult and usage for adolescents should not be generalized and should be used for those who are hyperlipidemic and monitored closely for growth[22].

2. INCREASED PHYSICAL ACTIVITY

Exercise programs like daily 30 minutes moderate intensity of physical activity which burns about 210 kcal/day approximately consuming 4-7 kcal/min have a valuable support

in reducing the cardiovascular risk. This is recommended by American Heart Association which is a reasonable and feasible approach to fitness therapy.

3. ELIMINATION OF ASSOCIATED RISK FACTORS

Increased alcohol consumption among our individuals is most common in increasing the cardiovascular risk. So, individuals who wish to drink should restrict their limit to 2 or fewer standard drinks per day. Also, individuals who present with increased triglyceride levels are strictly recommended to eliminate or decrease alcohol intake.

Another most common risk factor for cardiovascular risk is smoking. For smoking individuals, they are advised to quit smoking and the better way for the younger individuals are encouraged not to smoke. For individuals who are unable to quit smoking, various smoking cessation therapies are available which includes nicotine replacement therapy and drug therapy[23].

4. MEDICAL MANAGEMENT OF HYPERLIPIDEMIAS:

1. **HMG Co-A inhibitor**

Atorvastatin

Lovastatin

Fluvastatin

Rosuvastatin

Simvastatin

Pravastatin

2. **Lipoprotein lipase activator**

Bezafibrate

Clofibrate

Gemfibrozil

Fenofibrate

3. **Resins sequestrating bile acid**

Colestipol

Cholestyramine

Colesevelam

4. **Cholesterol absorption inhibitors**

Ezetimibe

5. **Triglyceride synthesis inhibitor**

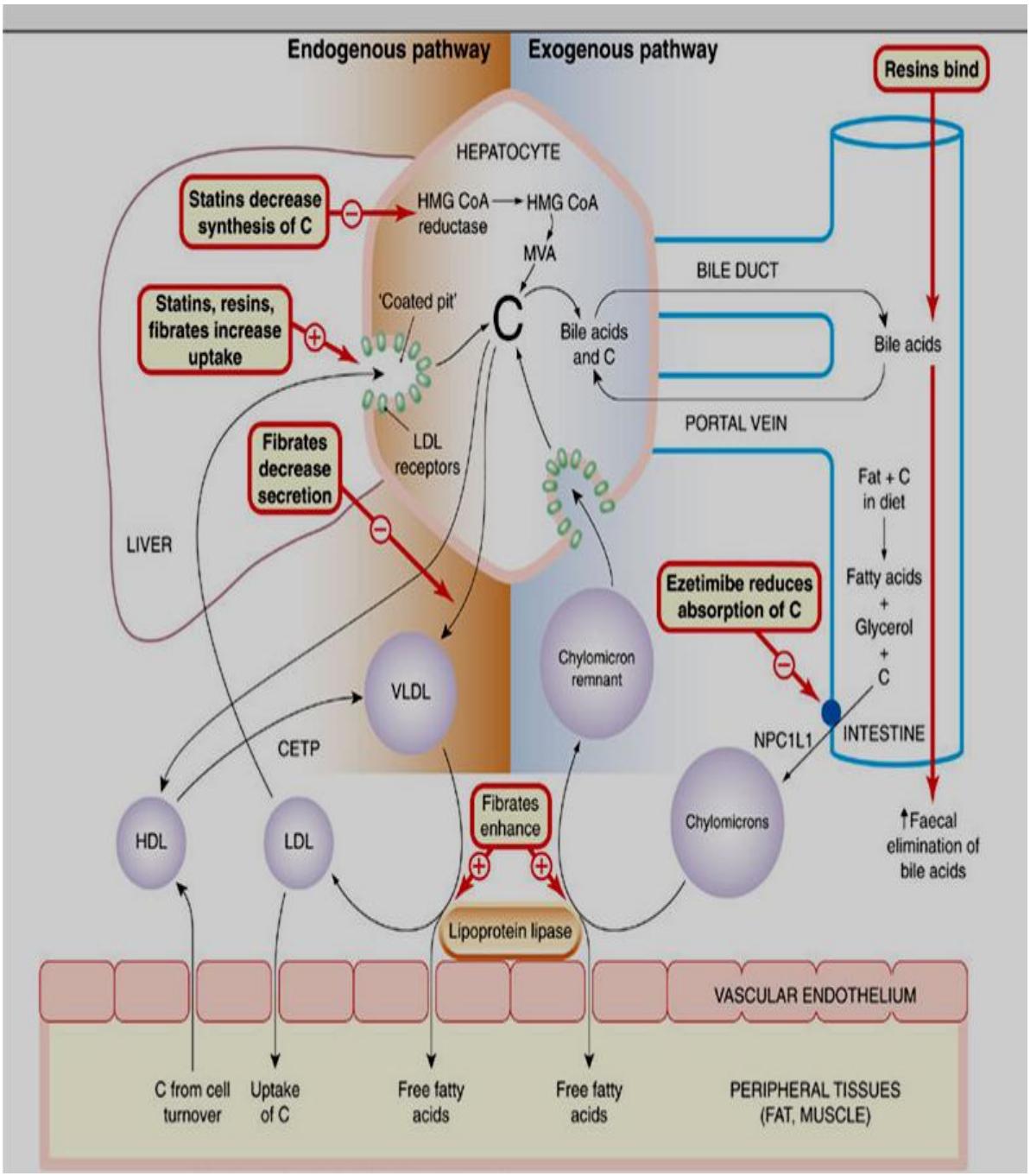
Nicotinic acid

6. **Miscellaneous**

Probucol[24]

Anti-oxidants – eg. Lycopene

Site of action of Statins, Fibrates, Ezetimibe and Resins used in Hypercholesterolemia



1. HMG-CO-A REDUCTASE INHIBITORS

ATORVASTATIN

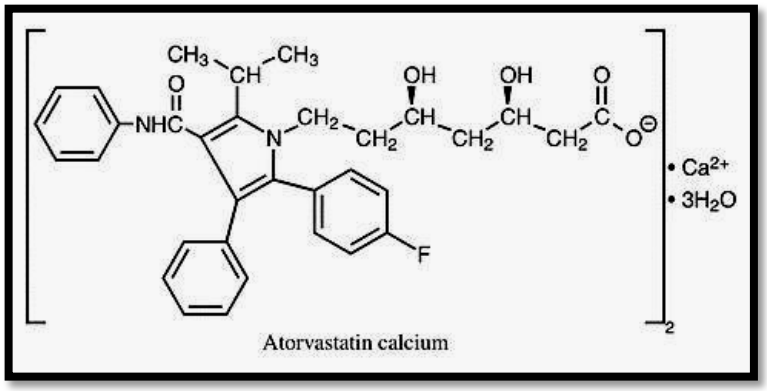
HISTORY:

In 1976, statins were first derived from a mould, *Penicillium citrinum*, and identified as inhibitors of cholesterol synthesis. Their mechanism of action is by inhibiting HMG-CoA reductase.

Compactin, later renamed as mevastatin was the first statin studied in humans. Lovastatin (formerly known as mevinolin) was the first developed statin and approved for use in humans, which was isolated from *Aspergillus terreus.* Six other statins are also available at present. The chemically modified derivatives of lovastatin are Simvastatin and Pravastatin. The structurally distinct synthetic compounds are atorvastatin, rosuvastatin, fluvastatin, and pitavastatin.

CHEMISTRY

- The structure of Atorvastatin is $(C_{33}H_{34}FN_2O_5)_2\,Ca_3H_2O$.
- Atorvastatin calcium is a white crystalline powder.
- In acidic pH, it is insoluble.



Atorvastatin calcium

MECHANISM OF ACTION

The major effects of statins are obtained by reducing LDL-C level through a mevalonic acid–like moiety which irreversibly inhibits the enzyme, HMG-CoA reductase. By inhibiting the conversion of pathway from HMG-CoA to mevalonate, which is the rate limiting step in the cholesterol synthesis, statins exert their inhibitory action at an early step.

In the hepatocytes, the decreased cholesterol synthesis results in transcription of genes for the synthesis of LDL receptors. The membrane bound protein called SREBPs are undergoes proteolysis and translocated to the nucleus. The transcription factor then binds with the sterol-response element of the LDL receptor gene results in increased transcription and synthesis of LDL receptors. Thus increased number of LDL receptors on the surface of hepatocytes results in increased removal of LDL-C from the blood by means of receptor mediated endocytosis, thereby lowering the blood LDL-C levels. The LDL receptors degradation is reduced. Statins also can reduce the LDL-C levels by enhancing the removal of precursors of LDL like VLDL and IDL. It also decreases the production of hepatic VLDL.

PHARMACOLOGICAL ACTIONS

1. Triglyceride levels

Statins are effective in reducing the triglyceride levels. The decrease in triglyceride levels achieved is similar to the level decreased in LDL cholesterol levels. The higher dose of most potent statins taken by hypertriglyceridemic patients observed a 35-45% decrease in LDL-C and an equal amount of reduction in fasting triglyceride levels is also noted.

2. HDL-C Levels

Statins shows a modest rise in HDL cholesterol levels. About 5-15% of increase is noted.

3. Effects of Statins on LDL-C Levels

Statins lower LDL-C upto 60%. The decrease in LDL cholesterol level is augmented with the addition of nicotinic acid in combination with statins.

4. Potential Cardio protective effects other than LDL-C lowering.

i. Action on endothelium

Vasoconstrictors and vasodilators play a dynamic role in the vascular endothelium in causing coronary heart disease. Statins plays an important role in endothelium by enhancing the production of a potent vasodilator Nitric oxide. Thus the vasodilating action improves the endothelial function and results in protection against cardiovascular risk.

ii. Plaque Stability

Statins inhibit infiltration of certain monocytes into the artery wall. They also inhibit secretion of matrix metalloproteinase (MMP) from the macrophages which normally degrades the extracellular matrix results in weakening of the fibrous cap. This will leads to the disruption of the atherosclerotic plaques. Thus by inhibiting MMP, prevent weakening of plaques that have formed. Other action in preventing atherosclerosis is by inhibiting smooth muscle cell proliferation and also by enhancing apoptosis.

iii. Anti- Inflammatory action

In coronary heart disease, the normal C- reactive protein levels will be elevated. Thus increase in C- reactive protein levels also said to be the risk factor for coronary heart disease. Recently, evidence has been shown proving this statement. Statins decrease the C- reactive protein levels. This is one of the anti-inflammatory actions noted in statins.

iv. Lipoprotein Oxidation

Oxidation of lipoproteins is the foremost mediating factor for the formation of atherosclerosis. The oxidized LDL cholesterol is taken up by the macrophages which results in the formation of foam cells. The oxidized lipoproteins also induce cytotoxicity inside the atherosclerotic lesions. Thus, statins reduce this oxidative reaction of lipoproteins and thereby decreasing the uptake of oxidized lipoproteins within the macrophages.

v. Coagulation

Statins mainly reduce the aggregation of platelets and reduce the adhesion molecules. Increased fibrinogen levels are associated with a greater in the incidence of cardiovascular risk.

- ❖ Atorvastatin has an additional anti-oxidant property.

PHARMACOKINETICS

Atorvastatin is rapidly absorbed from the stomach if administered orally. Atorvastatin has a longer plasma half life $t^{½}$ = 18 -24 hrs. It is > 98% bound to plasma proteins. It is

metabolized in the liver and the metabolites are important in exerting the HMG-Co-A inhibiting activity. It is eliminated primarily in bile.

USES

1. They are the first line drug for treatment of Hyperlipidemia with elevated LDL-C and total cholesterol levels.
2. It is also effective in secondary hypercholesterolemia eg: Diabetes and Nephrotic syndrome.
3. It also used in primary prevention of arterial disease in patients with risk factors for atherosclerosis.
4. It is used as a prophylaxis in the prevention of myocardial infarction and stroke.

ADVERSE EFFECTS

All statins are generally tolerated.

Notable side effects are:

1. Mild gastrointestinal complaints and headache.
2. Rise in serum transaminase level occasionally.
3. Muscle aches are the commonest side effect. Increase in CPK levels occur infrequently.
4. Myopathy is more common when other drugs are used concurrently like nicotinic acid / gemfibrozil or CYP3A4 inhibitor ketoconazole/erythromycin/cyclosporine.

2. **LIPOPROTEIN LIPASE ACTIVATOR**

Gemfibrozil, Benzafibrate and Fenofibrate cause reduction in VLDL levels.

MECHANISM OF ACTION

Fibrates are derivatives of isobutyric acid activating the enzyme lipoprotein lipase, most important in catabolism of VLDL. This results in decreasing levels of VLDL which ultimately results in lowering of TG level. The nuclear transcription gene regulating receptor, PPARα activation cause enhancement of lipoprotein lipase synthesis and fatty acid oxidation. PPARα also mediate the enhancement of LDL receptor expression in the hepatocytes.

The triglyceride levels are lowered about 20-50% and decrease in LDL cholesterol levels about 10-15%.

THERAPEUTIC USES

- It is the first line drug for patients with increased triglyceride levels.
- Acute pancreatitis is prevented in patients with severe hypertriglyceridemia and chylomicronaemia.

ADVERSE EFFECTS

Side effects are rare which includes gastrointestinal symptoms, rashes, impotence, myopathy, blurred vision, hypokalemia and increased blood levels of aminotransferases or alkaline phosphatase.

3. **RESINS SEQUESTRATING BILE ACID**

Cholestyramine, Colestipol and Colesevelam are the bile acid sequestrants useful only for patients with isolated increase in LDL.

MECHANISM OF ACTION

These drugs are the basic ion exchange resins available in the chloride form. After oral administration these drugs are neither digested nor absorbed in the stomach. It binds with the bile acids in the intestine and interrupts the bile acid entero-hepatic circulation. Faecal excretion of bile salts and cholesterol is increased. This indirectly leads to enhanced hepatic metabolism of cholesterol to bile acids.

ADVERSE EFFECTS

Flatulence and other gastrointestinal symptoms occur.

4. CHOLESTEROL ABSORPTION INHIBITORS[25]

Ezetimibe is a novel drug which acts by inhibiting cholesterol absorption from the intestine. It is rapidly absorbed from the gut and undergoes conjugation with the glucuronide in the intestine and excreted. The plasma half life was about 22 hours.

MECHANISM OF ACTION

Its main mechanism is interfering with a specific cholesterol transport protein called NPC1L1 inside the intestinal mucosa. This results in decreased absorption of both dietary and biliary cholesterol. The enhanced cholesterol synthesis can be blocked by statins, and the two drugs have synergistic LDL-CH lowering effect.

ADVERSE EFFECT

Reversible hepatic dysfunction and rarely myositis have been noted.

5. INHIBIT TRIGLYCERIDE SYNTHESIS AND LIPOLYSIS

Nicotinic acid is a B group vitamin, in higher doses decreases Triglycerides and VLDL-C rapidly followed by modest fall in LDL-C and Total Cholesterol. It is primarily excreted in the urine.

MECHANISM OF ACTION

The synthesis of triglyceride is inhibited by niacin which results in decreased release of VLDL in the liver. IDL and LDL are also reduced indirectly. It increases the activity of lipoprotein lipase that hydrolyse triglycerides.

USES

Combined with resins or statins, niacin reduces LDL cholesterol levels in heterozygous familial hypercholesterolemia patients.

ADVERSE EFFECTS

As nicotinic acid is a cutaneous vasodilator, marked flushing, itching occurs. This is associated with release of PGD_2 in the skin. Aspirin taken before niacin substantially attenuates flushing by inhibiting PG synthesis. Laropiprant is a specific anti-flushing drug with no hypolipidemic action has been combined with nicotinic acid to minimize flushing.

Dyspepsia, vomiting, diarrhea can occur. Dryness, hyper pigmentation of skin, liver dysfunction and jaundice, hyperglycemia, hyperuricaemia, atrial arrhythmia also occurs.

PROBUCOL

It is one of the anti-hyperlipidemic agent acts by inhibiting the cholesterol synthesis and cholesterol absorption. It also increases the rate of LDL degradation. It may also lower the HDL levels.

OXIDATIVE STRESS

It is defined as an ‘imbalance between free radical production and scavenging system predisposing to various pathological diseases’

‘A free radical (Reactive Oxygen Species) is defined as an atom with one or more unpaired electrons, capable of independent existence’

The reactive oxygen species (ROS) includes

- Superoxide anion radical ($O2^-$)
- Hydrogen peroxide ($H2O2^-$)
- Hydroxyl radical (OH^-)
- Nitro radical ($ONOO^-$)

Oxidative stress induced by reactive oxygen species (ROS) plays an important role in the etiology of several chronic diseases including coronary heart disease. Oxidation of the circulating LDL which carries cholesterol into the blood stream plays a key role in the pathogenesis of atherosclerosis.

ANTI-OXIDANTS[27]

'An anti-oxidant is a molecule stable enough to donate an electron to a free radical and neutralise it, thus reducing its capacity for tissue damage'

CLASSIFICATION 1

- ENDOGENOUS — Ubiquinone, glutathione, uric acid
- EXOGENOUS — Vitamin E, Vitamin C, Carotenoids (**Lycopene**)

CLASSIFICATION 2

- PRIMARY — Vitamin E, Vitamin C, carotenoids (Lycopene), ubiquinone, lipoic acid
- SECONDARY — N-Acetylcysteine, copper, glutathione reductase

USES:

Used in Hyperlipidemia, diabetes mellitus, hypertension, stroke, atherosclerosis, senility, auto-immune disorders, osteoarthritis, H. *pylori* infection, etc.,

Nutrients rich in antioxidants which have their ability to inhibit the oxidative process are believed to slow the progression of atherosclerosis. Thus antioxidants are the agents inactivating the ROS and therefore they significantly prevent the oxidative damage to the tissues.

Lycopene is one of the natural antioxidant which restores the endogenous antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), reduced glutathione (GSH), and glutathione reductase (GR).

In the previous study, Bose et al showed a significant increase in the level of antioxidant enzymes which demonstrated the antioxidant properties of tomato lycopene after 60 days of dietary supplementation of tomato in the coronary heart disease patients with respect to the control group and showed that it had a considerable therapeutic potential as an antioxidant[28].

LYCOPENE

Lycopene is a carotenoid phytonutrient without provitamin-A activity.

Lycopene is derived from the word neo-Latin ***lycopersicum*****,** the tomato species.

- **Other name:** rhodopurpurin
- **Scientific name:** non-provitamin A carotenoid.

It is present in many fruits and vegetables. It is a red, fat-soluble pigment found in certain plants and micro-organisms.

SOURCE

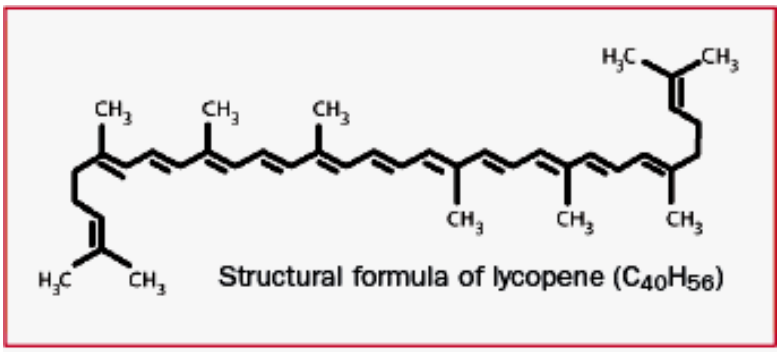
Major sources of lycopene in our diet includes

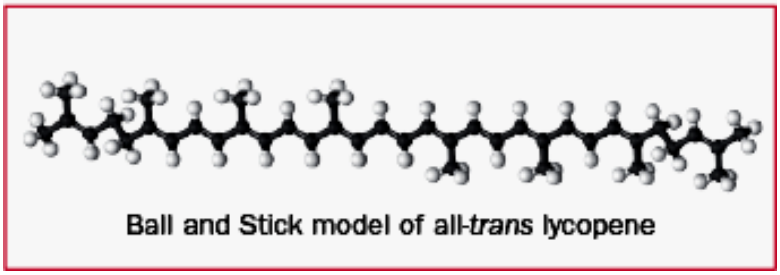
- Gac fruit (*Momordica cochinchinensis*),

- Tomatoes (*Lycopersicon esculentum*)
- Tomato products including tomato juice, ketchup, and pizza sauce.

- It is also found in watermelon, papaya, pink grapefruit, and pink guava[29].

STRUCTURE OF LYCOPENE





It is an isomer of β-carotene with an acyclic ring which contains 11 conjugated double bonds and 2 non-conjugated double bonds arranged in a linear array. Because of the presence of high number of conjugated dienes in their structure it is considered as the one of the most potent antioxidant.

Lycopene is mainly given importance these days due to its antioxidant property as many diseases have been found to be associated with decreased level of these antioxidants nowadays. It has a single oxygen quenching activity two times higher than β-carotene, 10 times higher than α-tocopherol signifying that lycopene has antioxidant property higher than these compounds[29].

BIOAVAILABILITY AND PHARMACOKINETICS

Lycopene is naturally present as all-trans form in its major sources like tomatoes. This form is poorly absorbed from the gut mucosa. Processing of tomatoes causes lycopene isomerization from its all-trans form to cis form which results in increasing bioavailability signifying that the processed forms are more useful for us[30].

High fat diet increases the absorption of lycopene as it is a fat soluble compound. The concentration of lycopene in body tissues is higher compared to other carotenoids.

In the small intestine it is incorporated with lipid micelles. These lycopene containing lipid micelles are solubilized in their hydrophobic form which helps to enter into the intestinal mucosal cells by means of passive diffusion. In the blood plasma, lycopene is taken up by the very low and low-density lipoprotein fractions.

Lycopene is widely taken up by various fatty tissues and to the organs such as the liver, adrenal glands, skin and testes. Lycopene levels are decreased with increasing age. But in individuals among smokers and alcoholics, the serum value of lycopene does not show any decreased levels[31].

The maximum concentration of lycopene after ingestion ranges from 15.6 to 32.6 h.The mean half-life of lycopene is between 28.1 and 61.6 h.

MECHANISM OF ACTION

The biological activities of carotenoids such as β- carotene are related in general to their ability to form vitamin A within the body[32].

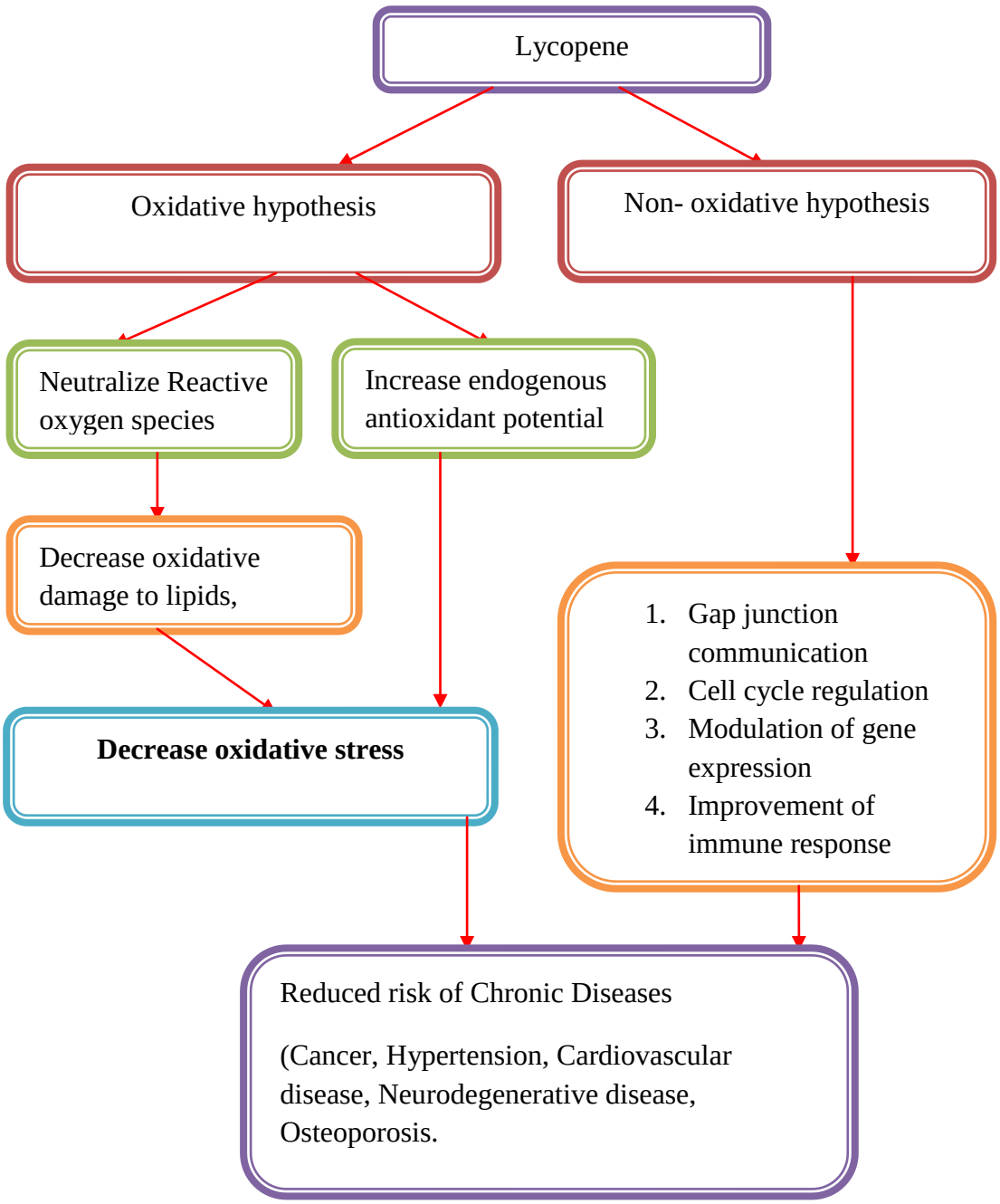
Although lycopene being an isomer of carotene, it cannot form vitamin A because of the lack of β-ionone ring in its structure. Thus the biological effects of lycopene in humans have therefore been attributed to mechanisms other than vitamin A.

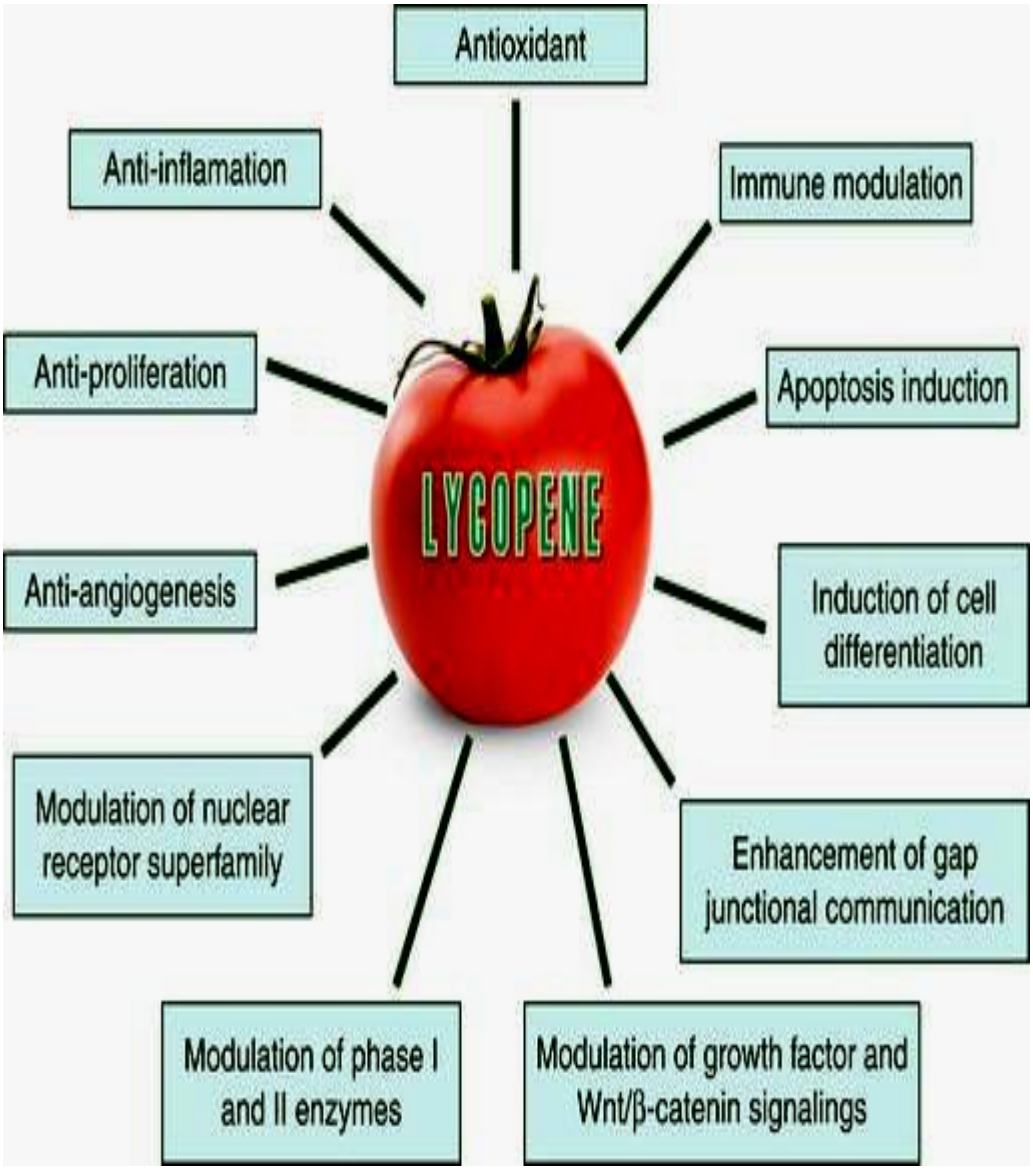
The presence of eleven conjugated double bonds in its structure has been attributed to its anti-oxidant property.

- ❖ Two major hypotheses have been proposed to explain the anti-carcinogenic and anti-atherogenic activities of lycopene:

1. Non-oxidative effects
2. Oxidative effects

1. The non-oxidative mechanism explains the anti-carcinogenic effect of lycopene. It primarily regulates the gap-junction communication between the cells demonstrated in the fibroblast cells of mouse embryo[33].
2. Oxidative mechanism – it protects LDL-C from oxidation and suppress cholesterol synthesis.
3. Hypocholesterolemic agent- inhibits 3-hydroxy-3-methylglutaryl–coenzyme A reductase enzyme.


Lycopene
Oxidative hypothesis
Non- oxidative hypothesis
Neutralize Reactive oxygen species
Increase endogenous antioxidant potential
Decrease oxidative damage to lipids,
1. Gap junction communication
2. Cell cycle regulation
3. Modulation of gene expression
4. Improvement of immune response
Decrease oxidative stress
Reduced risk of Chronic Diseases
(Cancer, Hypertension, Cardiovascular disease, Neurodegenerative disease, Osteoporosis.



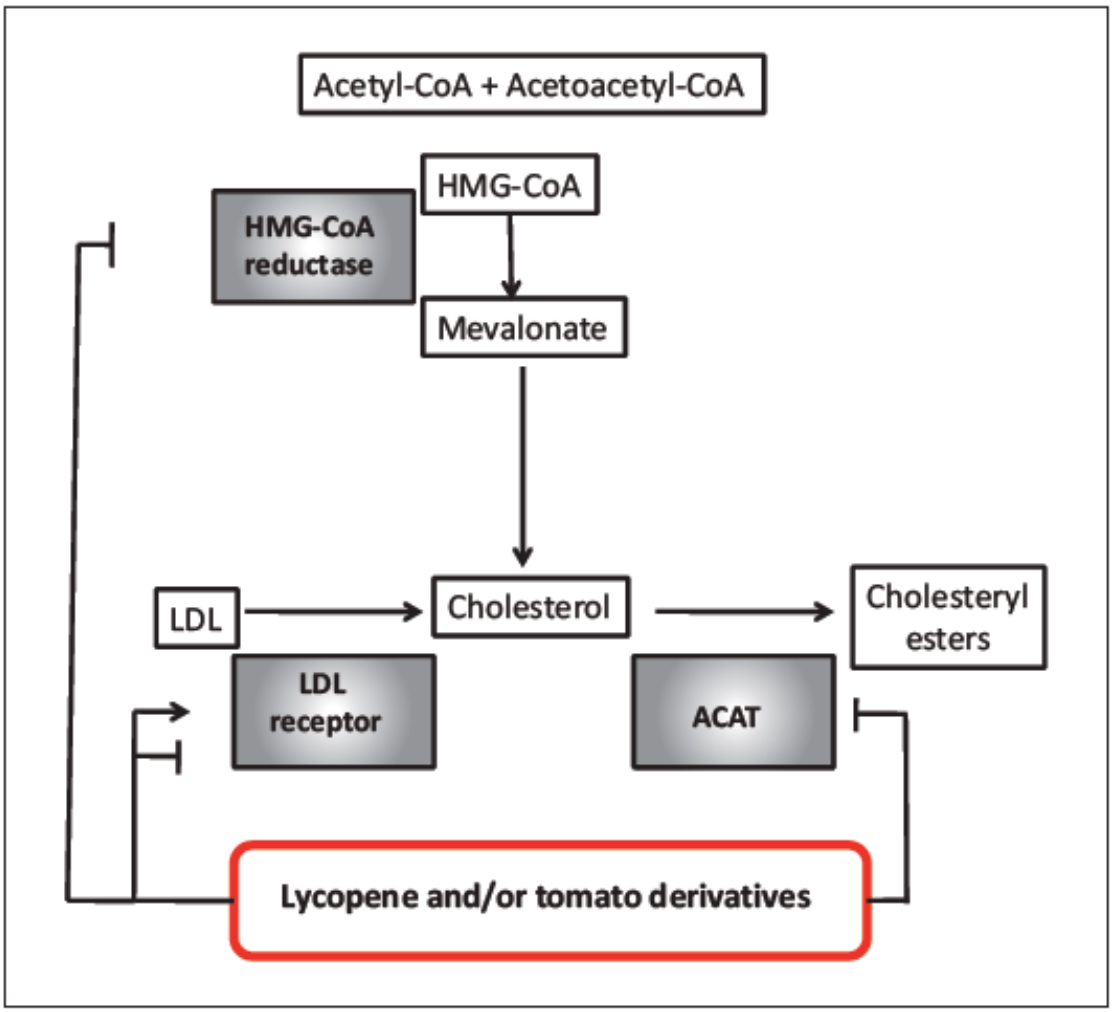
4. It protects various cellular bio molecules like lipids and lipoproteins which prevents the formation of atherogenesis. It also protects proteins and DNA molecules contributing to its anti-carcinogenic effect.
5. It suppresses phosphorylation of p53 and Rb anti-oncogenes which are common regulatory proteins affected by the carcinogens. It also stops cell cycle at the G0–G1 phase[34].

6. In the rat liver the carcinogen induced pre-neoplastic lesions are prevented by modulating the liver metabolizing enzyme, cytochrome $P_{450}2E_1$, this modulation helps in the protection against the carcinogen[35, 36].
7. In various cancers, insulin-like growth factors act as a potent mitogen causing proliferation of cells. Lycopene shows reduced cellular proliferation induced by insulin-like growth factor[37].
8. Suppression of mammary tumour growth by regulating intrathymic T-cell differentiation (immunomodulation)[38]

EFFECT OF LYCOPENE ON LIPID METABOLISM

Recently lycopene is also getting importance because of its effect on cholesterol metabolism and its long term effect on atherosclerosis. Following are the effects of lycopene on cholesterol metabolism[39, 40]:

1. The biosynthesis of cholesterol and isoprenoids is catalyzed by a specific rate limiting enzyme HMG-CoA reductase enzyme promoting deacylation of HMG-CoA to mevalonate.



2. Reduction of intracellular cholesterol by decreasing cholesterol synthesis through an inhibition of HMG-CoA reductase activity and expression, and modulation of LDL receptor and ACAT activity. The mechanism of HMG-CoA reductase enzyme is regulated by negative feedback mechanism by both the sterols and non-sterol products of the mevalonate pathway[41].
3. In J-774 A.1 macrophage cell line, lycopene suppress mainly synthesis of cholesterol from [3 H]-acetate, rather than from [14 C] mevalonate.
4. Lycopene reduces the serum cholesterol concentration by decreasing the cholesterol biosynthesis and also by enhancing the uptake through activation of the LDL receptors. Increased uptake of LDL by the macrophages enriched

with lycopene compared to the uptake of LDL by the cholesterol enriched macrophages has been noted[42].

5. Cholesterol regulates HMG-CoA reductase gene transcription. Statins competitively inhibits this enzyme whereas; lycopene inhibits this enzyme at the post transcriptional level.

Furhman et al, showed the effect of lycopene in six healthy male subjects consuming 60 mg/day of lycopene for three months duration. At the end of treatment period, a significant 14% reduction in their plasma LDL-C level was observed.

In postmenopausal women, supplementation with tomato extract capsules (4 mg lycopene) daily for 6 months duration also results in decreased total cholesterol and LDL cholesterol levels.

EFFECT OF LYCOPENE IN ATHEROGENESIS

1. In atherogenesis, the various inflammatory mediators like tumor necrosis factor (TNF-α) and interleukin (IL)-1β, and IL-8 enhance the binding of low-density lipoprotein to endothelial surface and enhance the up-regulation and expression of leukocyte adhesion molecules on the endothelium[43]. These inflammatory mediators play a major role in atherogenesis. Low density lipoproteins are the main carrier of cholesterol into the blood stream. Thus the oxidation of the low density lipoproteins forming oxidized LDL (LDLox) is thought to play an important role in the atherogenesis. Atherosclerosis is the underlying disorder causing cardiovascular events like heart attack and stroke.
2. Thus lycopene exerts its anti-atherogenic effect at the level of these inflammatory mediators preventing the formation of atherogenesis. It also has an additional

effect in inhibiting TNF-α-induced NF-κB activation, monocyte-endothelial interaction and ICAM-1 expression in human umbilical endothelial cells.

3. Lycopene also suppresses the transcription reactions TNF-α-induced IκB phosphorylation, NF-κB expression, and NF-κB p65 translocation from the cytosol to nucleus[44].
4. Thus, in the prevention of atherosclerosis, lycopene shows significant reduction in the levels of oxidized LDL (LDLox), thereby reducing the level of cholesterol in the blood stream[45].

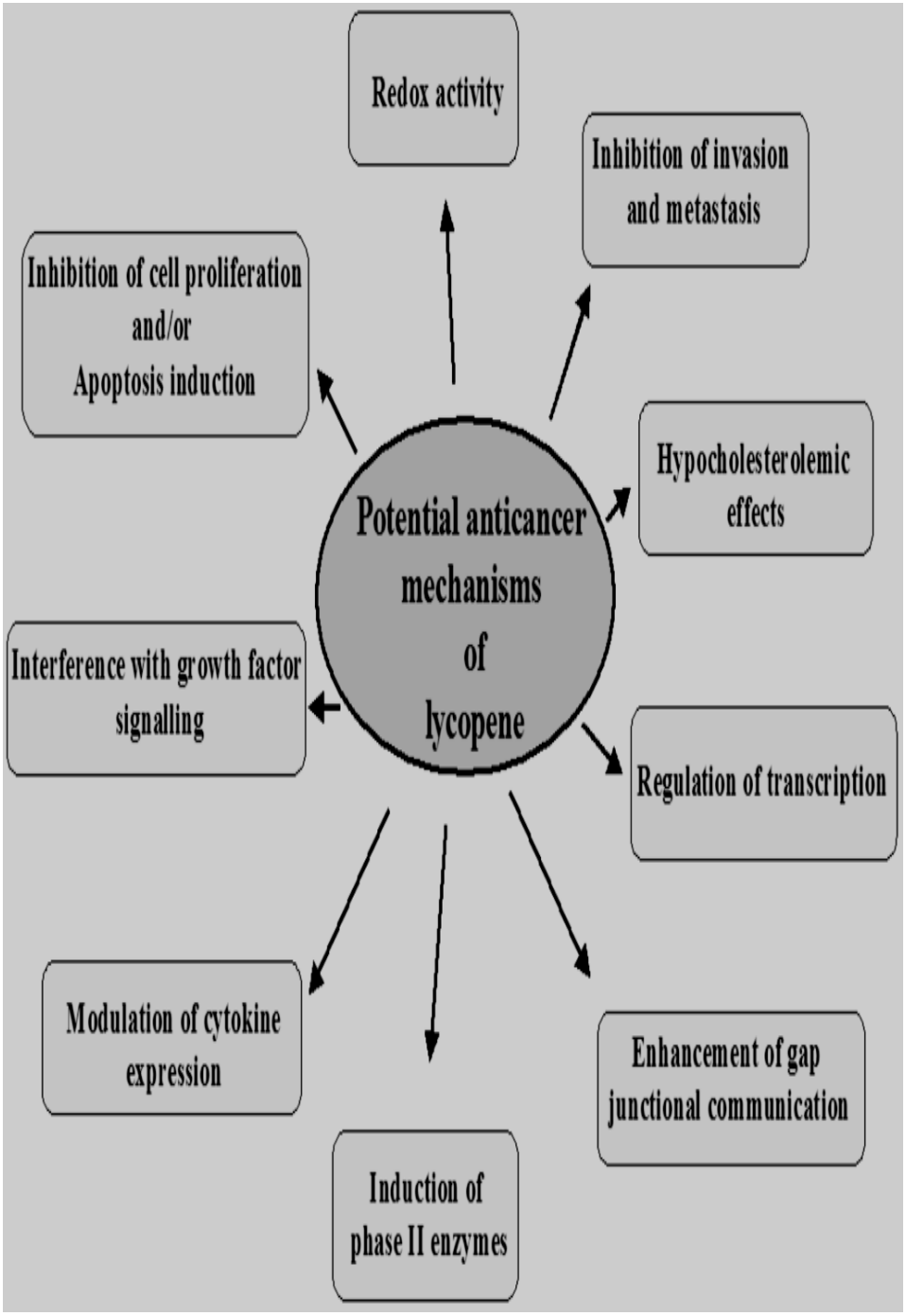
In a study by Visioli et al, 12 females were allowed to consume tomato products like raw tomatoes, sauce and paste for three weeks duration. In these individuals it shows increased lycopene concentration in their blood and also reduced oxidizability of LDL cholesterol levels. This concluded that lycopene present in the tomato products decreases lipid peroxidation, thereby protects from atherosclerosis[46].

EFFECT OF LYCOPENE ON PREVENTING CANCER

1. Increased intake of lycopene shown to reduce the incidence of various cancers like cancers of the colon, pancreas, rectum, esophagus, oral cavity, cervix and breast [47].
2. In oxidative mechanism, it shows to protect the cellular biomolecules like proteins and DNA from oxidative damage. Thus the oxidative damage to DNA was reduced and shows 20% significant decline in incidence of prostate cancer[48].
3. Lycopene decreases cellular proliferation in various cell lines. By down-regulating the PI-3K/Akt/mTOR signaling pathway and decreased proliferation of HT-29 cells in the human colon results in suppression of colon cancer[49].

4. In human cultured skin fibroblast, it binds with the platelet –derived growth factor-BB. This results in inhibition of platelet-derived growth factor-BB–induced signaling and cell migration[50].
5. In prostate cancer cell lines, lycopene enhance the induction of apoptosis and it also inhibits the cell growth in androgen-sensitive (LNCaP) and androgen-independent (PC3 and VeCaP) cells[51].
6. In old age men, daily intake lycopene containing 15 mg/day shown to inhibits the progression of benign prostate hyperplasia[52].
7. A gene called Connexin 43 is present in both human and animal cells. In many human tumors, there is a defect in the intercellular gap junction which causes increased proliferation of cancer cells. Lycopene up regulates the expression of this gene resulting in restoration of intercellular gap junction communication and associated with decreased proliferation of cancer cells[53].

Anticancer mechanism of lycopene



EFFECT OF LYCOPENE ON OSTEOPOROSIS

1. Oxidative stress and antioxidants play a major role in the pathogenesis of osteoporosis[54].
2. Endogenous antioxidants decrease the effects of stress induced by reactive oxygen species in these cells, thus lycopene decrease the damaging effects of oxidative stress.
3. Lycopene has bone remodelling effect by increasing the bone mineral density primarily by increasing the alkaline phosphatase of osteoblasts. It also has anti-resorptive property by inhibiting the osteoclast formation and maturation[55].
4. Lycopene also decreases the level of resorptive elements like N-telopeptides of type I collagen (NTx) [56].

MALE INFERTILITY

Infertility in males is a common reproductive disorder occurs due to the oxidative damage of the sperm. Lycopene being an antioxidant property it shows benefits in sperm morphology, sperm motility, sperm motility index, and functional sperm concentration. The effective response is observed by continuous intake of lycopene for 12 months duration[57].

EFFECT OF LYCOPENE ON PROTECTION OF HEPATIC DAMAGE

Hepatic damage occurs by necrosis of hepatocytes, increase in tissue lipid peroxidation, and depletion of tissue endogenous antioxidant enzymes like GSH levels. The various serum biochemical markers like serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, triglycerides, cholesterol, bilirubin, and alkaline phosphatase are elevated when hepatic damage is present[58].

In an animal model, the protective effect of lycopene was demonstrated. A rat with galactosamine/lipopolysaccharide (D-GalN/LPS)-induced hepatitis was injected lycopene intraperitonealy a dose of 10 mg/kg. The serum concentration of biochemical markers are shown to be reduced in this animal model.

The decrease in biochemical markers like triglyceride, cholesterol and free fatty acids showed a protective effect in hepatic damage[59].

In another study it was demonstrated that lycopene significantly restored the endogenous antioxidant liver enzymes, such as glutathione peroxidase, glutathione-s-transferase, which is protective in many disease caused by oxidative stress[60].

EFFECT ON EYE DISORDERS

Cataract is the most common multifactorial disorder. It usually occurs in elderly people. In diabetic individuals, increased osmotic stress and decreased antioxidant defense mechanisms are contributes to the changes observed in the eye predisposing to the formation of cataract. In various epidemiological studies, the nutritional antioxidants decrease the progression of cataract formation and age-related macular degeneration[61].

In an in vitro study, lycopene protects the human retinal pigment epithelium cell line ARPE-19 against the H_2O_2-induced oxidative stress[62].

OTHER DISEASES

1. Lycopene is a fat soluble compound which crosses the blood brain barrier. Human brain is a common organ for affected by oxidative damage due to it's increased uptake of oxygen and utilization. Brain also possesses low antioxidant effects. In normal individuals low concentration of lycopene is present in the brain which has a protective effect.
2. Lycopene is decreased in elderly individuals and it shows that there is significant reduction in the lycopene levels in most common diseases like Parkinson's disease and vascular dementia[63].
3. Lycopene was also suggested as providing protection against amyotrophic lateral sclerosis (ALS) disorder in humans[64].
4. It also shows a protective role in neurodegenerative diseases like Alzheimer'sdisease[65].
5. Future research will also explore the importance of lycopene in other human diseases including rheumatoid arthritis, diabetes, ocular and skin disorders, periodontal diseases and inflammatory disorders[66].

RECOMMENDED DAILY ALLOWANCE

- In healthy individuals, daily intake of 5-7 mg/day of lycopene helps in combating oxidative stress and prevention of chronic illness.
- 15-20 mg/day of Lycopene shows significant effects on LDL oxidation, blood pressure, platelet activation and aggregation and DNA oxidative damages.

DRUG FORMULATIONS

It is available as

- Soft gel capsules

- Syrup

ADVERSE EFFECTS

Anorexia,

Diarrhoea,

Flatulence,

Nausea,

Vomiting,

Skin discolouration,

Abdominal pain.

DRUG INTERACTIONS

1. May increase the risk of bleeding with aspirin, anticoagulants, heparin, anti-platelet drugs, NSAIDs.

PRECAUTIONS

1. Persons who are allergic or sensitive to Lycopene.
2. Pregnancy and breast feeding.

Lycopene by acting as an antioxidant can prevent the progression of many diseases at an early stage and improve the quality of life.

Lycopene with antioxidant effect helps in lowering blood lipid levels and may be beneficial in patients with hyperlipidemia. Since few studies are available on lipid lowering effect of lycopene, this study has been undertaken to assess the hypolipidemic effect in comparison with Atorvastatin.

AIM & OBJECTIVES

AIM

- To evaluate the efficacy and tolerability of Lycopene as add on therapy to Atorvastatin in reducing the lipid levels in patients with hyperlipidemia.

OBJECTIVES

Primary objective:

- Decrease in Total cholesterol and LDL cholesterol levels.

Secondary objective:

- Increase in HDL, decrease in VLDL, TGs.
- Observe the adverse effects.

METHODOLOGY

METHODOLOGY

This prospective study was done to assess the therapeutic effect of Lycopene in combination with Atorvastatin in decreasing the lipid profile.

STUDY DESIGN

This study was an open label, randomized, comparative, prospective study.

STUDY CENTRE

Department of Internal Medicine, Rajiv Gandhi Government General hospital, Madras Medical College, Chennai.

STUDY PERIOD

The study was carried out from August 2013 to April 2014 with 8 weeks as treatment period every patient and follow-up every fortnightly till the end of the study.

STUDY POPULATION

Patients presenting with Hyperlipidemia attending Medicine OPD, RGGGH/MMC, Chennai.

SAMPLE SIZE

- Totally 100 patients
- 50 patients in each group (control and test groups)

STUDY PROCEDURE:

The study was started after obtaining approval from the Institutional Ethics Committee. The patients were explained about the details of the study and an

information sheet and informed consent forms written in the regional language was provided to each patient and the patients willing to participate in this study signed the required forms.

INCLUSION CRITERIA:

1. Both genders.
2. Age- 25 – 60 yrs.
3. Subjects with total cholesterol level between 200-250 mg/dl.
4. Patients with Stage I Hypertension and Type II Diabetes with glycaemic control.
4. Patients willing to give written informed consent.

EXCLUSION CRITERIA:

1. Pregnant and lactating women.
2. Subjects with evidence of clinically significant gastrointestinal, renal, respiratory, endocrine, hematological, neurological, psychiatric or cardiovascular dysfunctions.
3. Triglycerides > 250 mg/dl.
4. Total cholesterol: HDL ratio > 4.5.
5. H/o allergy or intolerance to lycopene.
6. Patients unwilling or unable to comply with the study procedures.
7. Those with history of alcohol or drug abuse.

SCREENING

The patients were screened with detailed clinical history, physical examination and baseline investigations.

RECRUITMENT

Those who fulfilled the inclusion criteria were recruited for the study.

RANDOMIZATION:

The enrolled patients were randomized to the control or test group by simple randomization.

TREATMENT PLAN

CONTROL GROUP

T. Atorvastatin 10mg/day for 8 weeks.

TEST GROUP

T. Atorvastatin 10mg/day and Capsule Lycopene 15mg/day for 8 weeks.

The study medication was issued for 2 weeks. After assessing the compliance at the end of 2 weeks, study medication was issued for the subsequent 2 weeks. The same procedure was followed till the completion of study.

INVESTIGATION:

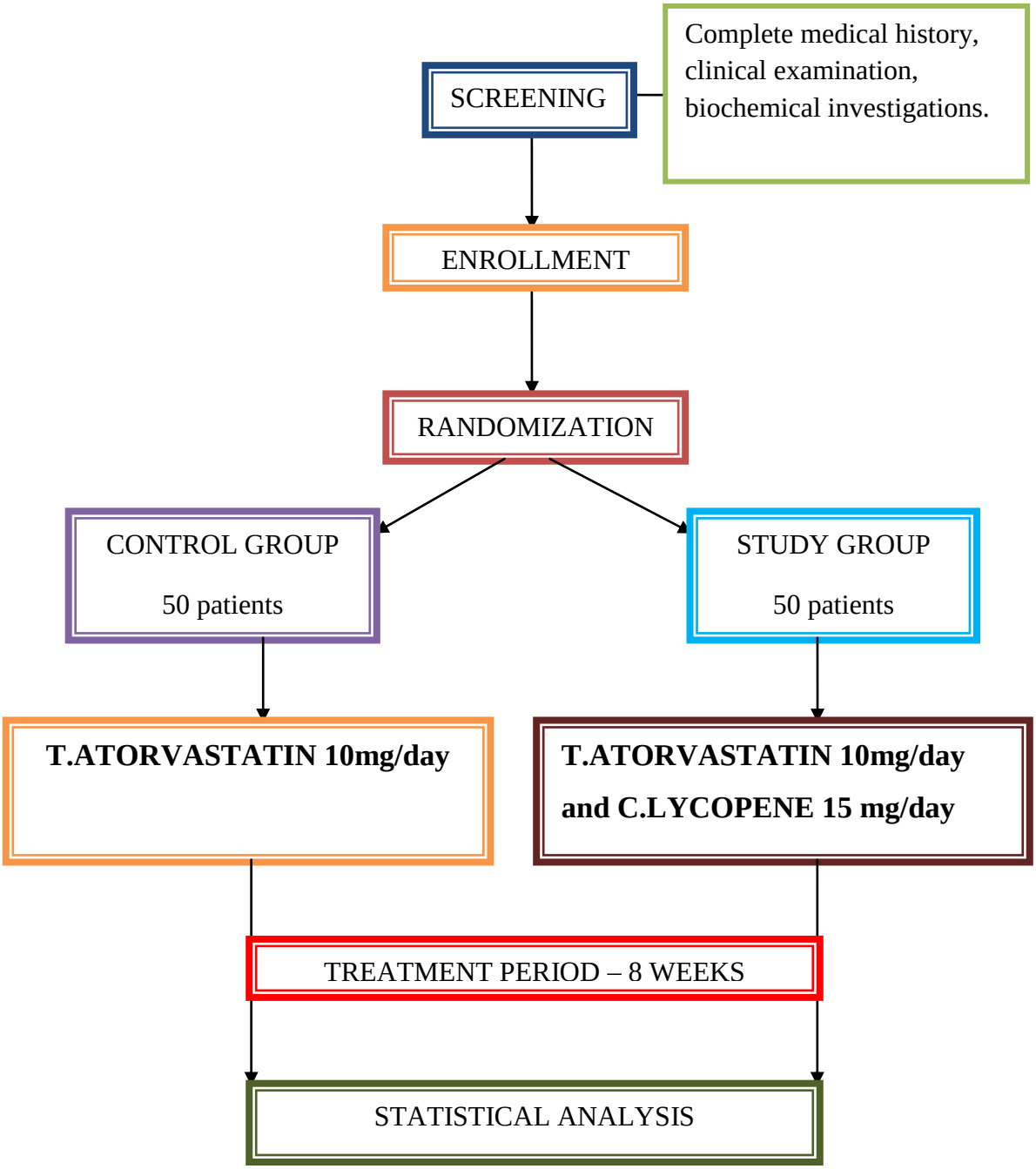
BASELINE INVESTIGATION

- Complete blood count (Hemoglobin, Total count, Differential count, ESR and Platelets)
- Fasting Blood sugar, Blood urea, Serum Creatinine.
- SGOT, SGPT
- Routine Urine Analysis

All the baseline investigations were done at the start of the study and at the end of the 8 weeks.

- Fasting Lipid Profile: Serum (Total Cholesterol, LDL, VLDL, HDL & TGL) was done at baseline, 4^{th} week and at 8^{th} week of the study.

STUDY FLOW CHART



STUDY VISITS

Screening and Baseline

1. Demographic details obtained
2. Complete medical history recorded
3. Clinical examination performed
4. Enrollment done
5. Written informed consent obtained
6. Vitals recorded
7. Laboratory investigation
    - Complete blood count
    - Lipid profile
    - Blood urea, Sugar
    - Serum Creatinine
    - SGOT, SGPT
    - Urine analysis

VISIT 1 (0 WEEKS)

1. Randomization of patient
2. Physical & Clinical examination
3. Vitals recorded
4. Drugs issued for Study group and control group patients
5. Instruction to return the empty strips in the subsequent visit.

VISIT 2 (2 WEEKS)

1. Received Empty drug strips
2. Clinical examination was done
3. Vitals recorded

4. Drugs issued for subsequent 2 weeks
5. Instruction to return the empty strips in the subsequent visit.
6. Patient advised to report any adverse event.

VISIT 3 (4 WEEKS)

1. Received Empty drug strips
2. Clinical examination was done
3. Vitals recorded
4. Drugs issued for subsequent 2 weeks.
5. Lipid profile
6. Instruction to return the empty strips in the subsequent visit.
7. Patient advised to report any adverse event

VISIT 4 (6 WEEKS)

1. Received Empty drug strips
2. Clinical examination was done
3. Vitals recorded
4. Drugs issued for subsequent 2 weeks
5. Instruction to return the empty strips in the subsequent visit.
6. Patient advised to report any adverse event.

VISIT 5 (8 WEEKS)

1. Received Empty drug strips
2. Clinical examination was done
3. Vitals recorded
4. Laboratory investigation
    - Complete blood count

- Lipid profile
- Blood urea, Sugar ,Serum Creatinine
- SGOT, SGPT
- Urine analysis

INSTRUCTION TO PATIENTS

The patients were instructed clearly regarding the regular intake of the medicines. They were given proper advice to report for assessment and collection of drugs. They were counseled to report any adverse reactions occur.

COMPLIANCE

Patient compliance was monitored by the empty strips returned at each visit.

ADVERSE EVENTS:

Adverse event if any, reported by the patient or observed by the physician during the study was recorded. The onset of adverse event, causal relationship to the study drug and action taken was recorded.

STATISTICAL ANALYSIS:

The obtained data was analyzed statistically. Distribution of age was analysed using ANOVA and Sex distribution was analyzed by Chi square test.

The biochemical investigations were performed at 0 week and 8^{th} week. The difference within the groups before and after treatment was analyzed using student's paired t-test whereas the difference between the Control and Study group were analyzed using students independent t-test.

p value < 0.05 is considered to be statistically significant.

RESULTS

RESULTS

This study was conducted to evaluate the effect of lycopene in combination with atorvastatin in reducing lipid levels.

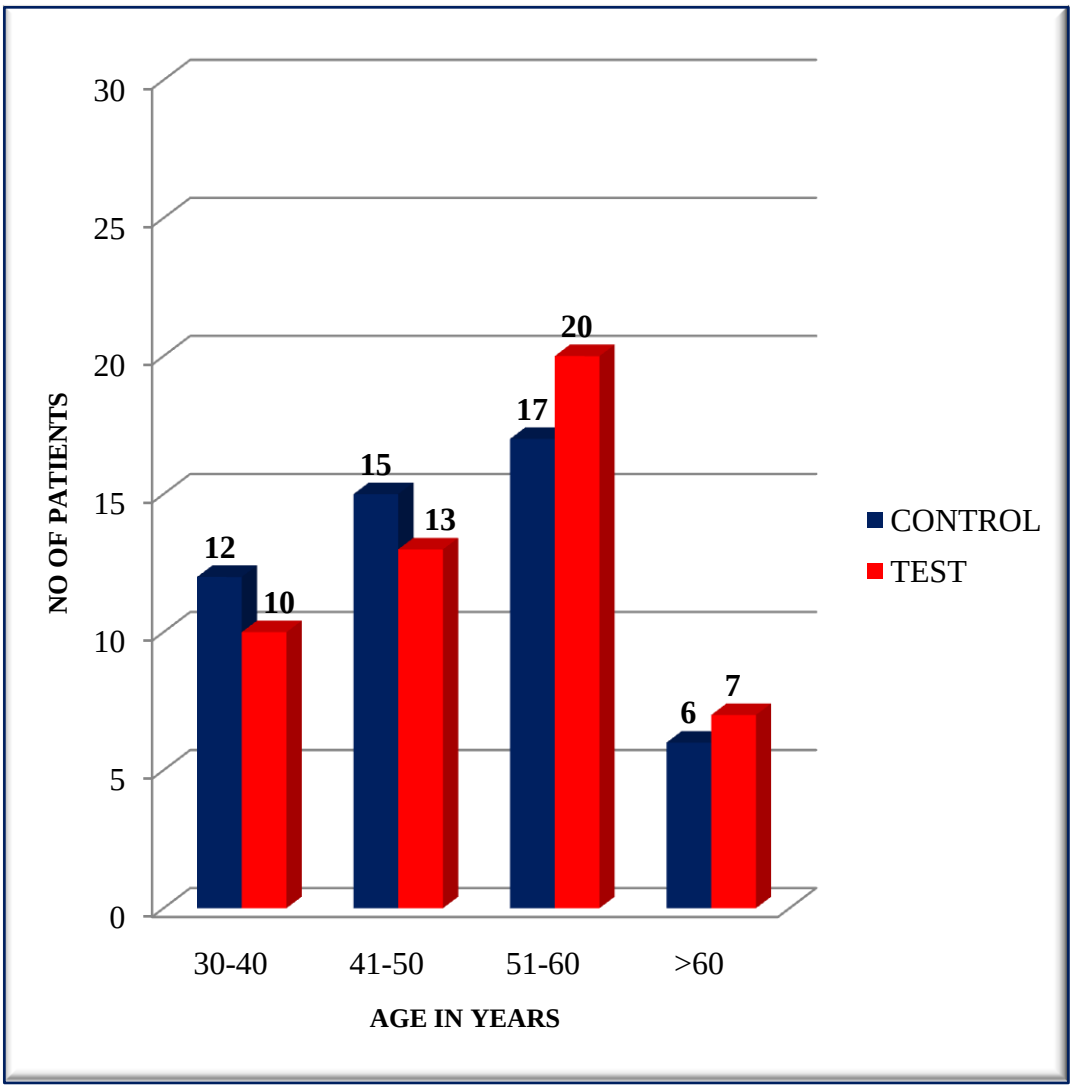
- 176 patients were screened
- 60 patients were excluded from the study based on exclusion criteria,
- 16 patients who were eligible for the study were not willing to participate.
- 100 patients were enrolled and completed the study.
- There were no drop outs.

TABLE 1: AGE DISTRIBUTION

AGE IN YEARS	CONTROL		TEST	
	NO	PERCENTAGE	NO	PERCENTAGE
30-40	12	24%	10	20%
41-50	15	30%	13	26%
51-60	17	34%	20	40%
>60	6	12%	7	14%
TOTAL	50	100%	50	100%

- Table 1 shows the age distribution of both the study groups.
- Age group 51-60years had more number of patients followed by age group 41-50.

FIGURE 1: AGE DISTRIBUTION



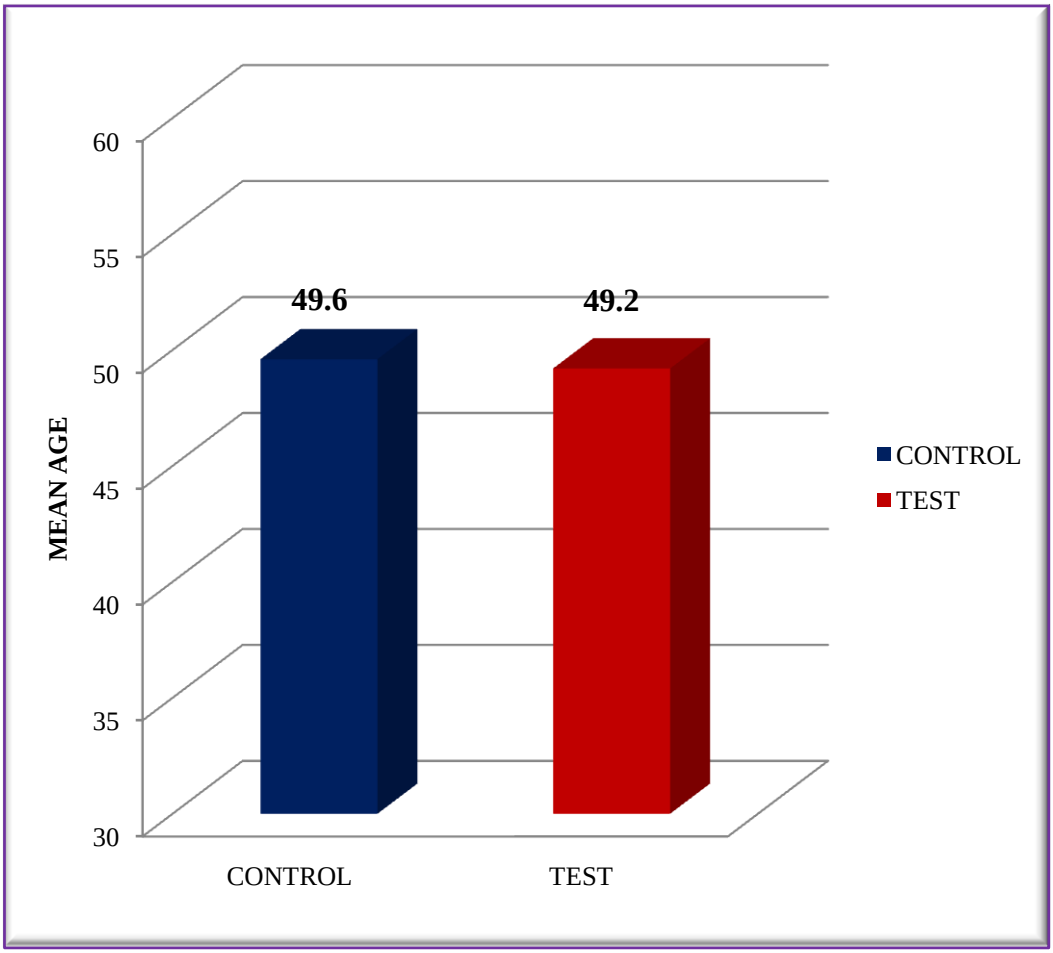
❖ Figure 1 depicts age distribution in both the study groups.

TABLE 2: MEAN AGE DISTRIBUTION

GROUP	No of patients	MEAN AGE (in years)	SD
Control	50	49.6	12.65
Test	50	49.2	12.41
p value		**0.893**	

- Table 2 shows the mean age of both the study groups.
- The mean age was similar in both the groups.
- There was no statistically significant difference between the groups.

FIGURE 2: MEAN AGE DISTRIBUTION



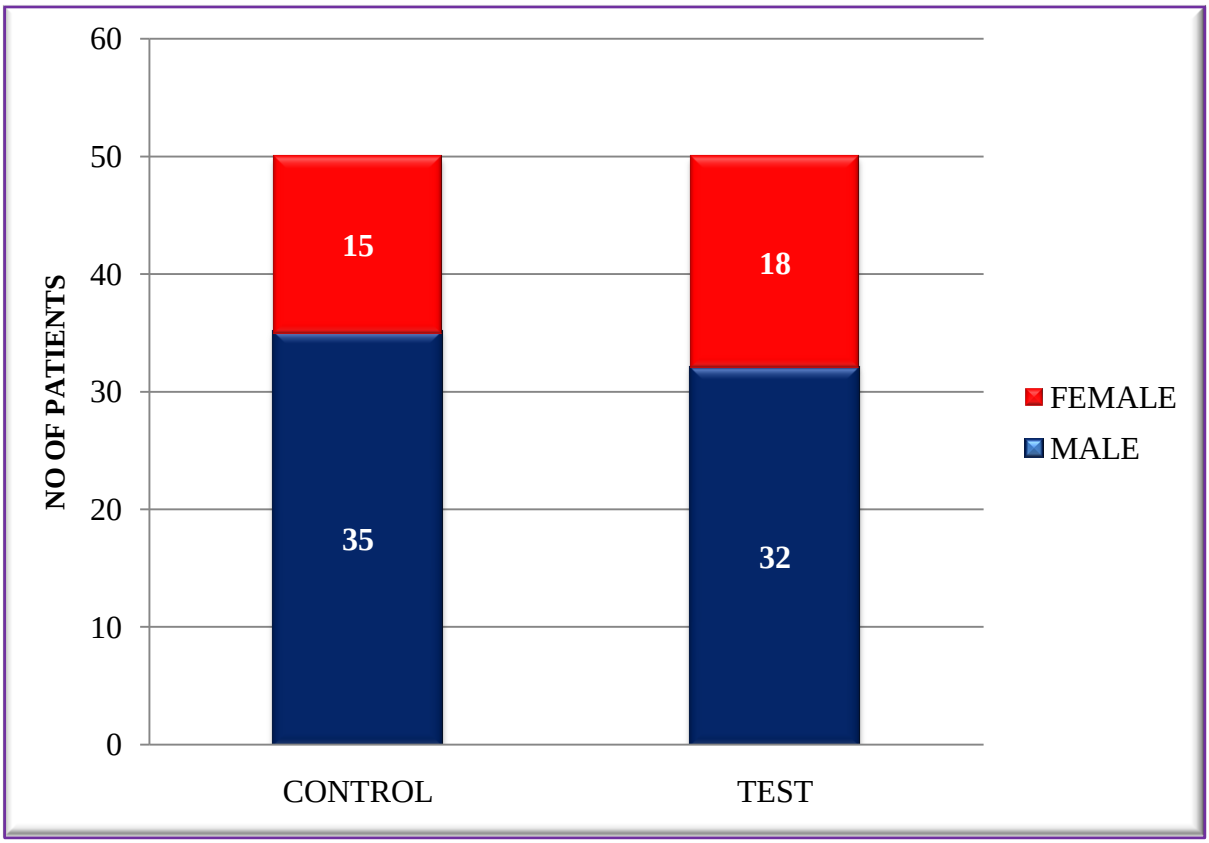
❖ Figure 2 is the graphical representation of Table 2.

TABLE 3: SEX DISTRIBUTION

SEX DISTRIBUTION	CONTROL		STUDY	
	NO. OF PATIENTS	%	NO. OF PATIENTS	%
MALE	35	70%	32	64%
FEMALE	15	30%	18	36%
TOTAL NO. OF PATIENTS	50		50	

- Table 3 shows the sex distribution in both the groups.
- Males were more in number compared to females in both groups.

FIGURE 3: SEX DISTRIBUTION



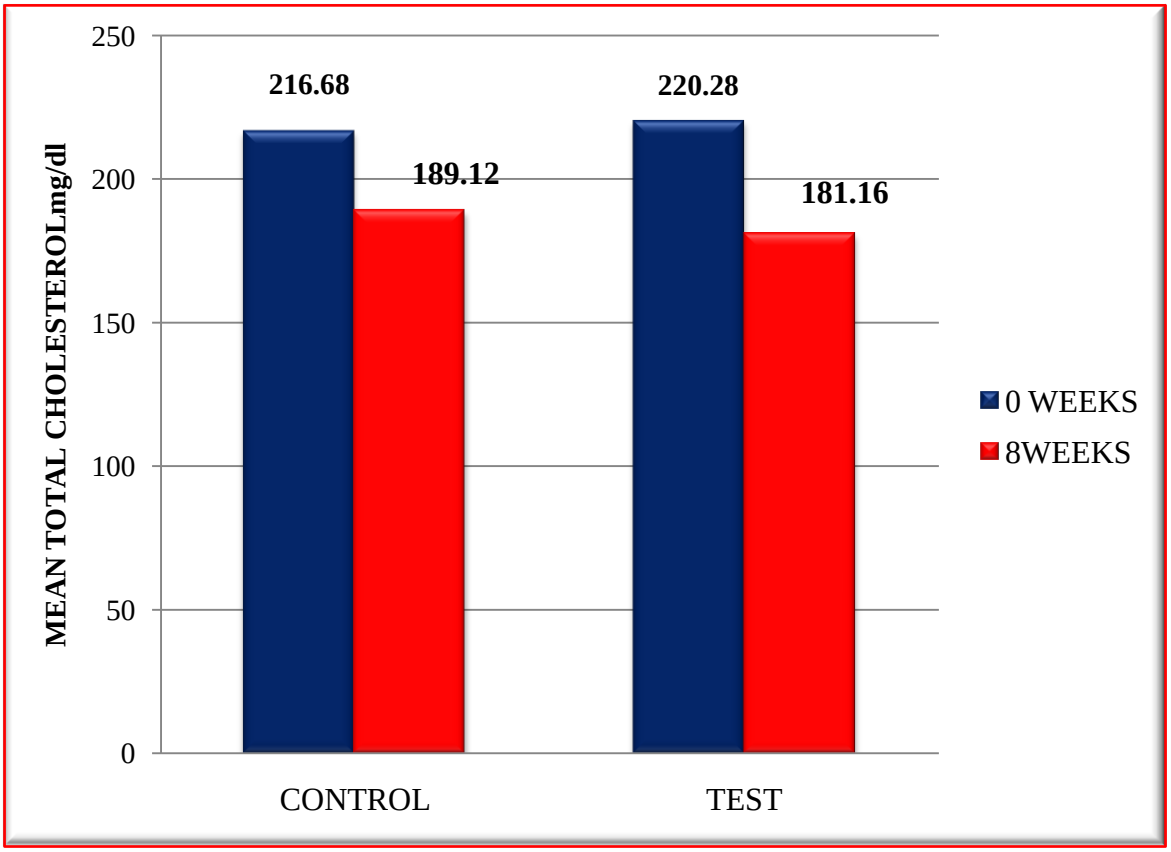
❖ Figure 3 depicts Table 3.

TABLE 4: TOTAL CHOLESTEROL

GROUPS	0 WEEKS		8 WEEKS		p value
	MEAN	SD	MEAN	SD	
CONTROL	216.68	15.94	189.12	10.87	**<0.001**
TEST	220.28	16.78	181.16	11.34	**<0.001**
p value	0.274		**0.01**		

- Table 4 shows the mean Cholesterol value of both study groups at baseline & end of 8 weeks.
- On comparing with baseline, both groups showed a decrease in mean Total cholesterol.
- Statistical analysis within the groups showed a significant decrease in the Total cholesterol at the end of 8weeks ($p < 0.001$).
- Statistical analysis between the groups showed a significant difference at the end of 8 weeks ($p = 0.01$).

FIGURE 4: TOTAL CHOLESTEROL



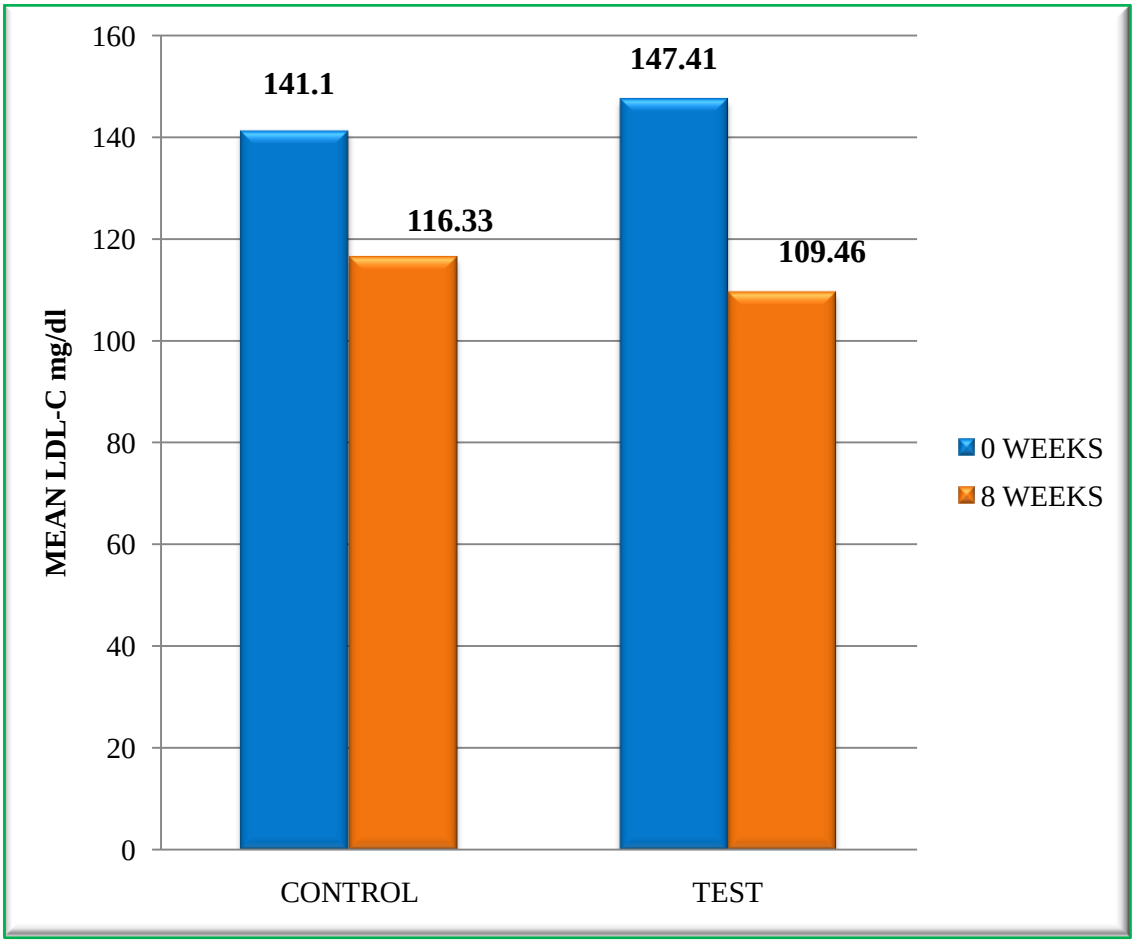
❖ Figure 4 is the graphical representation of Table 4.

TABLE 5: LDL CHOLESTEROL

GROUPS	0 WEEKS		8 WEEKS		p value
	MEAN	SD	MEAN	SD	
CONTROL	141.1	15.92	116.33	11.79	**<0.001**
TEST	147.41	2.70	109.46	1.63	**<0.001**
p value	0.08		**0.004**		

- Table 5 shows mean LDL cholesterol levels of both the groups at baseline & end of 8 weeks.
- There was a significant reduction in the mean LDL cholesterol levels in the test group (109.46mg.dl) compared to the control group (116.33mg/dl) at the end of 8 weeks.
- Statistical analysis within the group showed a significant decrease in both the study groups ($p < 0.001$)
- Statistical analysis between the groups at the end of 8 weeks showed a significant difference.

FIGURE 5: LDL CHOLESTEROL



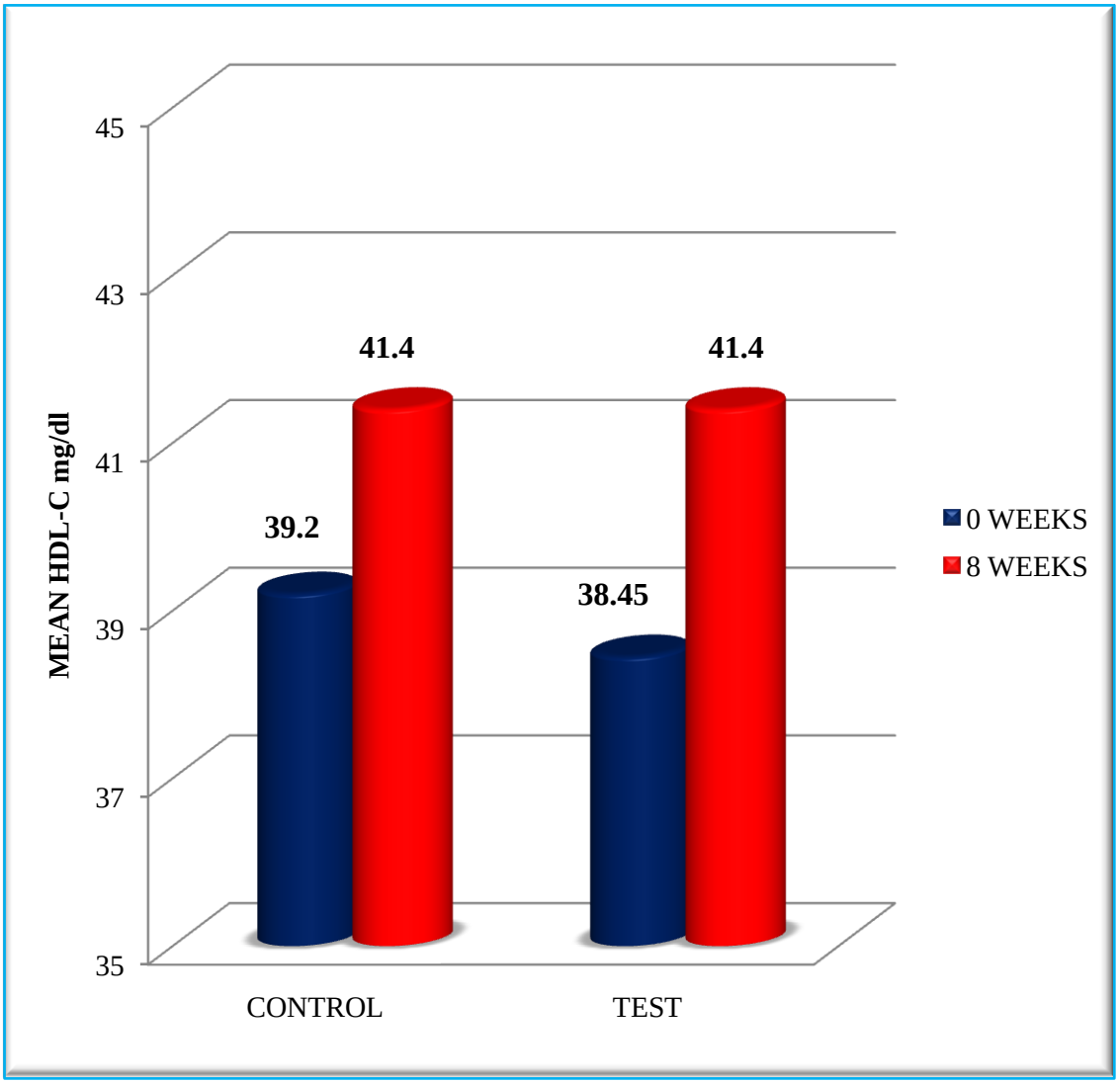
❖ Figure 5 depicts Table 5.

TABLE 6: HDL CHOLESTEROL

GROUPS	0 WEEKS		8 WEEKS		p value
	MEAN	SD	MEAN	SD	
CONTROL	39.2	1.76	41.4	1.64	**<0.001**
TEST	38.45	2.11	41.40	2.13	**<0.001**
p value	0.059		**0.958**		

- Table 6 shows mean HDL cholesterol levels in both the groups at baseline & end of 8 weeks.
- Statistical analysis within the groups showed a significant decrease in the HDL level at the end of 8 weeks ($p < 0.001$).
- Statistical analysis between the groups did not show a significant difference at the end of 8 weeks.

FIGURE 6: HDL CHOLESTEROL



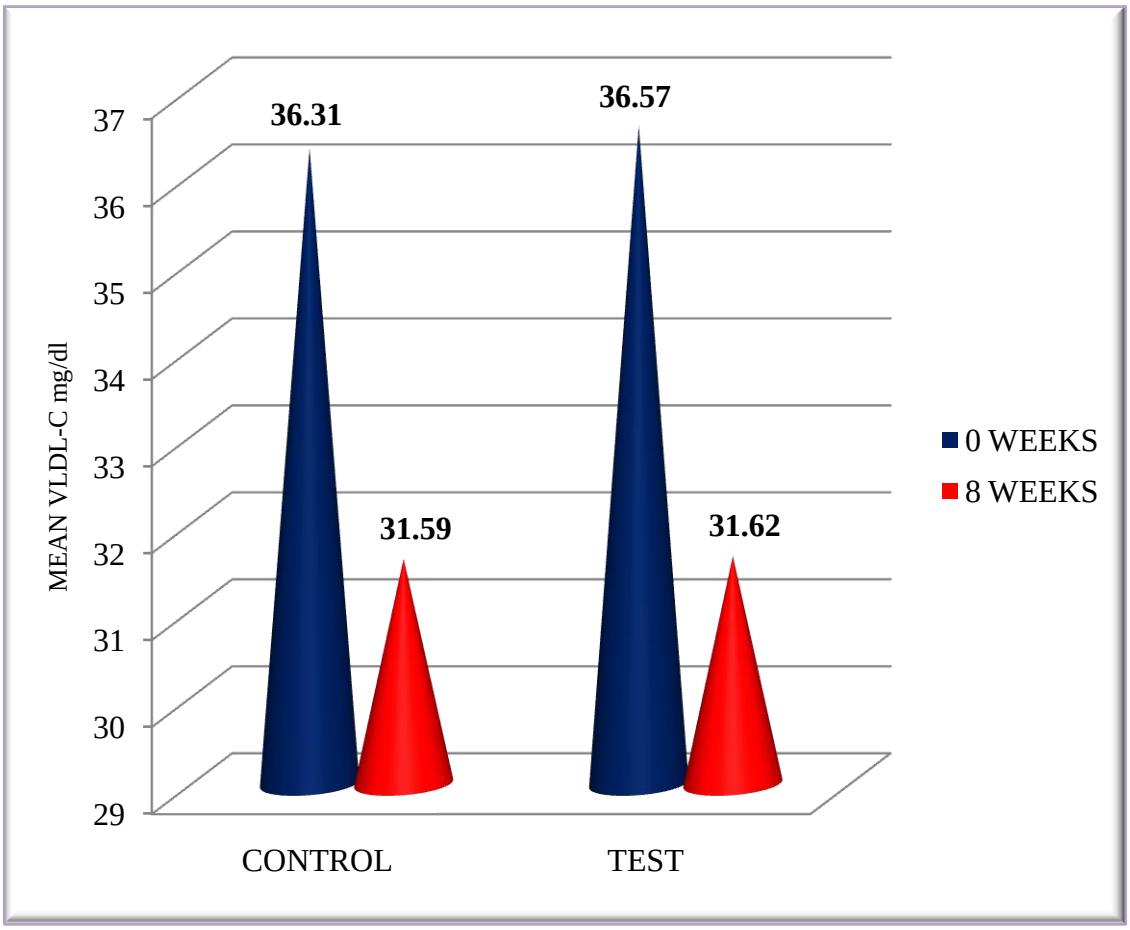
❖ Figure 6 is the diagrammatic representation of Table 6.

TABLE 7: VLDL CHOLESTEROL

GROUPS	0 WEEKS		8 WEEKS		p value
	MEAN	SD	MEAN	SD	
CONTROL	36.31	3.18	31.59	1.50	**<0.001**
TEST	36.57	3.6	31.62	1.69	**<0.001**
p value	0.704		**0.921**		

- Table 7 shows mean VLDL levels in both groups at Baseline and at the end of 8 weeks.
- Statistical analysis within the groups showed a significant decrease in the VLDL level at the end of 8 weeks ($p < 0.001$).
- Statistical analysis between the groups did not show a significant difference at the end of 8 weeks.

FIGURE 7: VLDL CHOLESTEROL



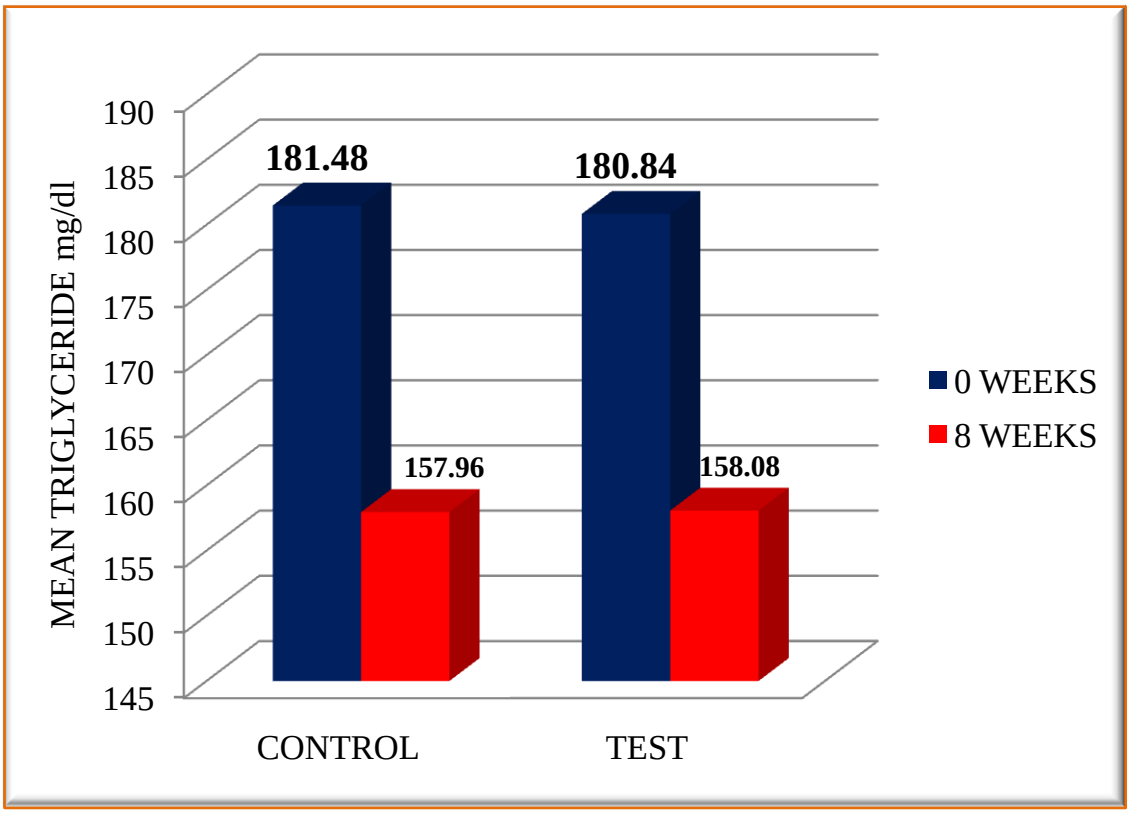
❖ Figure 7 depicts Table 7.

TABLE 8: TRIGLYCERIDE LEVELS

GROUPS	0 WEEKS		8 WEEKS		p value
	MEAN	SD	MEAN	SD	
CONTROL	181.48	15.93	157.96	7.54	**<0.001**
TEST	180.84	21.4	158.08	8.48	**<0.001**
p value	0.866		**0.941**		

- Table 8 shows mean Triglyceride level in both groups.
- Statistical analysis within the groups showed a significant decrease in the Triglyceride level at the end of 8 weeks ($p < 0.001$).
- Statistical analysis between the groups did not show a significant difference at the end of 8 weeks.

FIGURE 8: TRIGLYCERIDE LEVEL



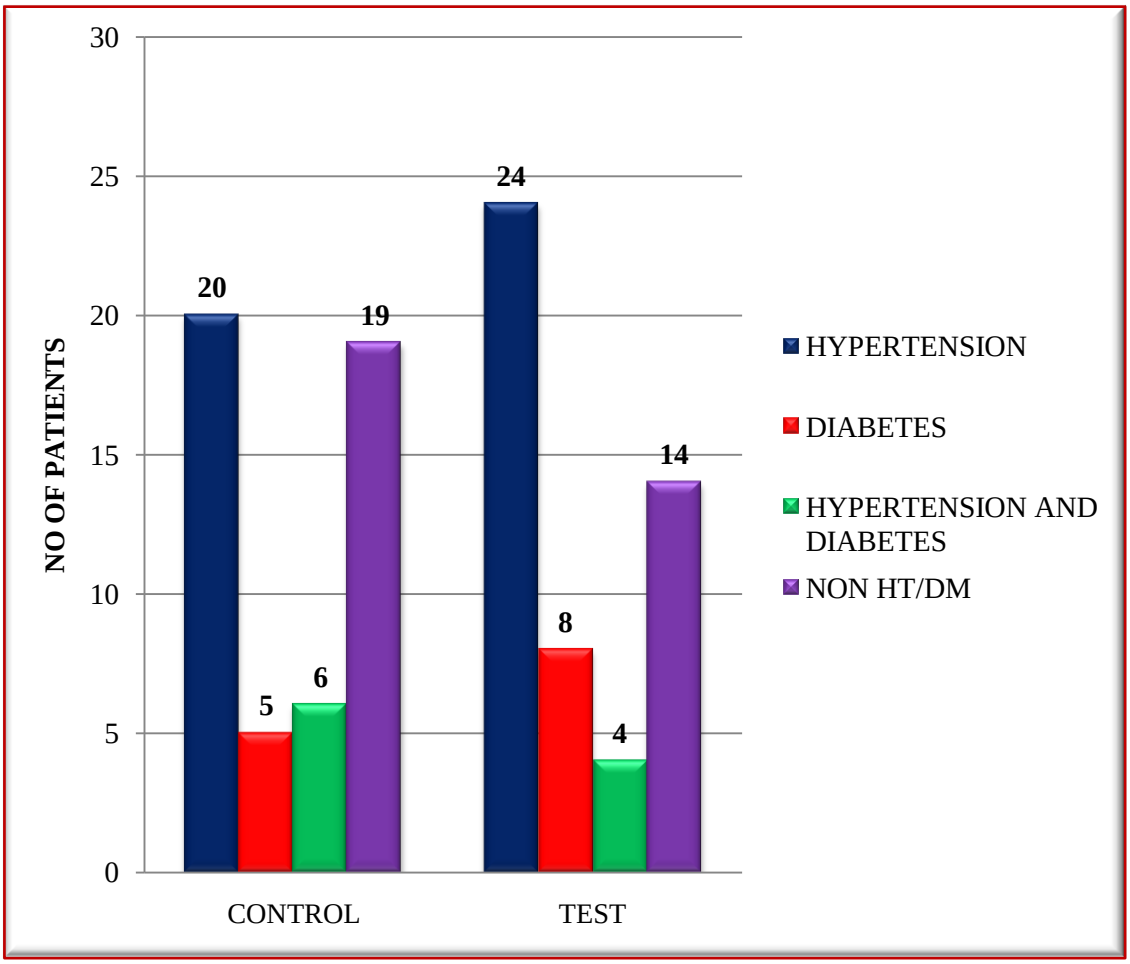
❖ Figure 8 is the graphical representation of Table 8.

TABLE 9: HYPERTENSION AND DIABETES

ASSOCIATED CONDITIONS	CONTROL	TEST	TOTAL
HYPERTENSION	20	24	44
DIABETES	5	8	13
HYPERTENSION AND DIABETES	6	4	10
NON HT/DM	19	14	33
TOTAL	50	50	100

- Table 9 shows the associated conditions in patients of both the study groups.
- Patients with Hypertension were more in both groups (Control group – 20, Test group – 24) followed by Non DM/HT (Control group-19, Test group-14).

FIGURE 9: HYPERTENSION AND DIABETES



- Figure 9 is the diagrammatic representation of associated conditions in patients of both the study groups.

TABLE 10: BIOCHEMICAL INVESTIGATION (CONTROL GROUP)

Investigations	Control group		p value
	0 WEEKS	8 WEEKS	
Blood urea	24.88±3.37	24.46±3.24	0.528
Blood sugar	103.10±19.5	100.7±8.93	0.634
Serum Creatinine	0.65±0.13	0.67±0.14	0.545
SGOT	28.26±4.66	27.62±4.54	0.561
SGPT	30.32±4.97	30.16±4.90	0.90
Total count	8188.8±1411.90	8159±1411.1	0.221
Hemoglobin	11.48±1.00	11.73±1.02	0.303

- Table 10 shows the Biochemical and Hematological parameters of the control group.
- Statistical analysis within the groups and between the groups did not show any significant difference.

TABLE 11: BIOCHEMICAL INVESTIGATION (TEST GROUP)

Investigations	Test group		p value
	0 WEEKS	8 WEEKS	
Blood urea	24.86±3.45	25.11±3.48	0.696
Blood sugar	107.6±19.68	104±12.27	0.754
Serum Creatinine	0.68±0.14	0.67±0.14	0.744
SGOT	27.92±4.33	28.46±4.37	0.588
SGPT	30.30±4.72	30.04±4.71	0.770
Total count	8309.5±1438.7	8274.7±1407.6	0.456
Hemoglobin	11.68±1.09	11.75±1.24	0.220

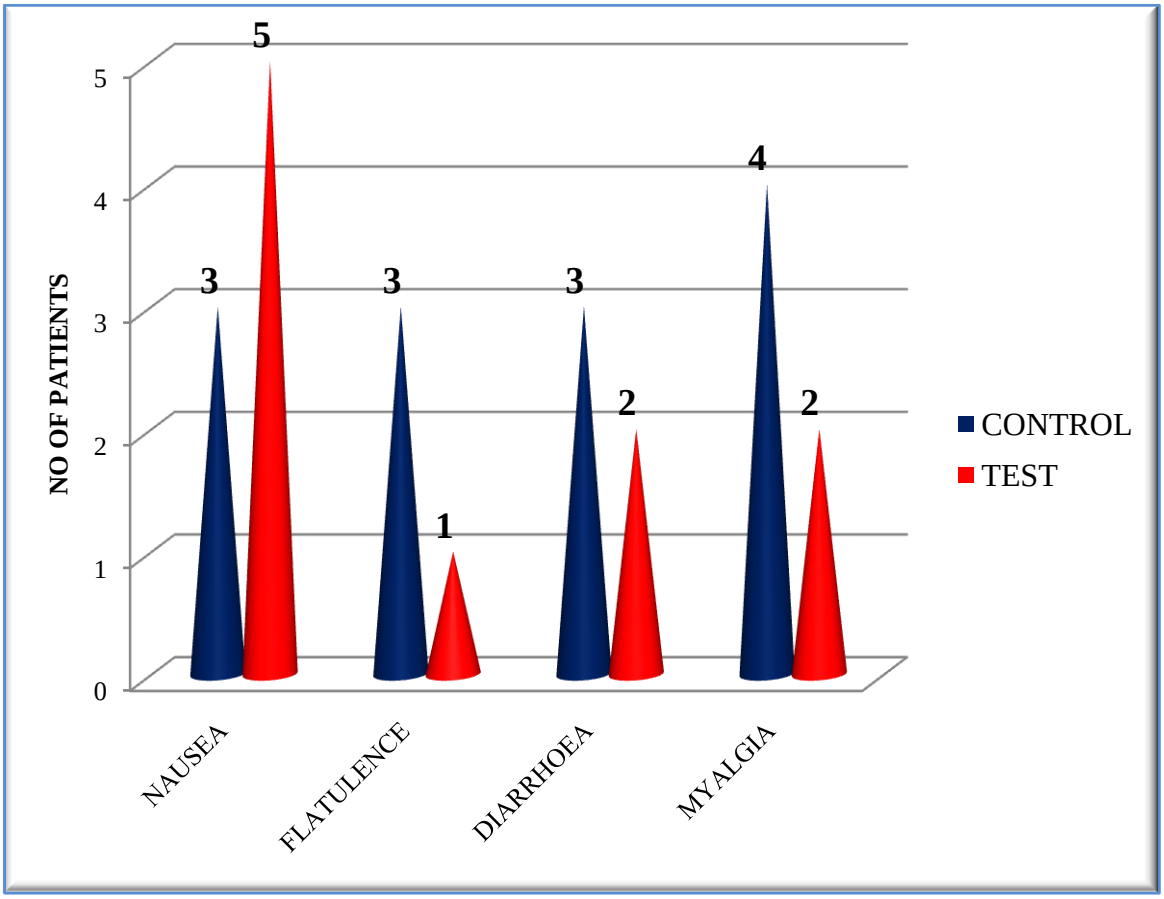
- Table 11 shows the Biochemical and Hematological parameters of the test group.
- Statistical analysis within the groups and between the groups did not show any significant difference.

TABLE 12: ADVERSE DRUG REACTION

ADVERSE DRUG REACTION	CONTROL GROUP	TEST GROUP
Nausea	3 (6%)	5 (10%)
Flatulence	3 (6%)	1 (2%)
Diarrhoea	3 (6%)	2 (4%)
Myalgia	4 (8%)	2 (4%)

- Table 12 shows the adverse effect profile of both the study groups.
- Gastrointestinal disturbances were reported more in both the study groups.

FIGURE 12: ADVERSE DRUG REACTION



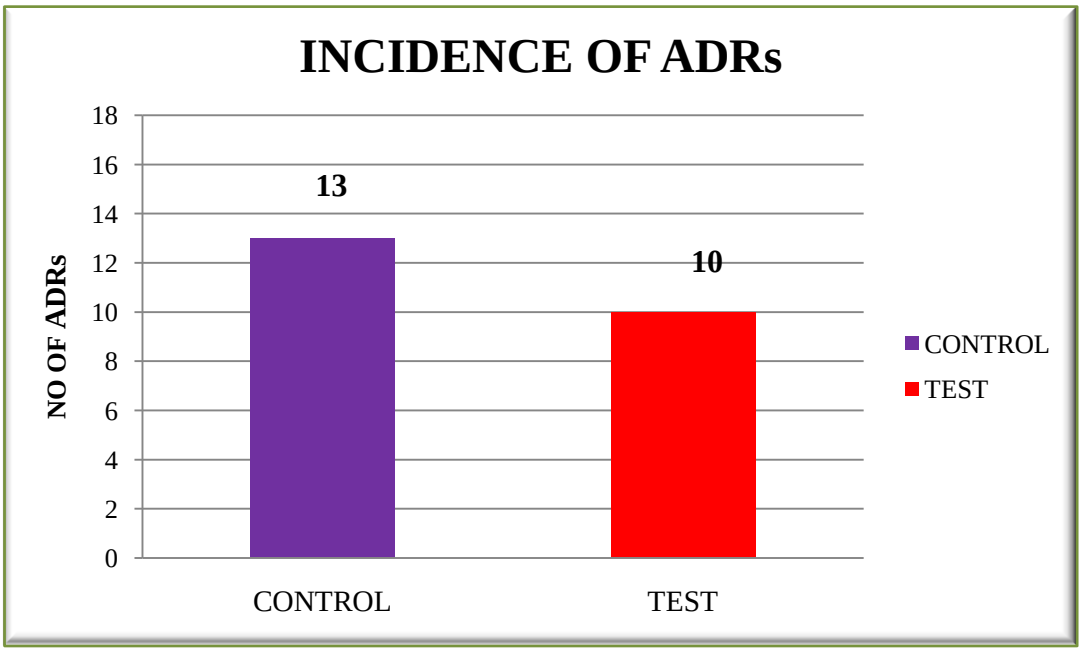
❖ Figure 12 is the graphical representation of adverse drug reaction.

TABLE 13: INCIDENCE OF ADRs

	CONTROL GROUP	TEST GROUP
NUMBER OF ADRs	13	10

- Table 13 shows the incidence of ADRs presented by the patients in both the study groups.
- In control group, 13 ADRs were reported and in Test group 10 ADRs were reported.

FIGURE 13: INCIDENCE OF ADRs



- Figure 13 shows the graphical representation of incidence of ADRs.

TABLE 14 : CAUSALITY ASSESSMENT OF INDIVIDUAL ADR IN CONTROL GROUP

ADRs	Certain	Probable	Possible	Un-likely	Un-classified	Un-classifiable	Total
Nausea	**-**	**-**	**3**	**-**	**-**	**-**	**3**
Flatulence	**-**	**-**	**3**	**-**	**-**	**-**	**3**
Diarrhoea	**-**	**-**	**3**	**-**	**-**	**-**	**3**
Myalgia	**-**	**-**	**4**	**-**	**-**	**-**	**4**
Total			**13**				**13**

- Table 14 shows causality assessment of individual ADR in control group.
- Causality assessment was done using WHO causality assessment scale
- All adverse drug reactions were categorized as possible.

TABLE 15 : CAUSALITY ASSESSMENT OF INDIVIDUAL ADR IN TEST GROUP

ADRs	Certain	Probable	Possible	Un-likely	Un-classified	Un-classifiable	Total
Nausea	**-**	**-**	**5**	**-**	**-**	**-**	**5**
Flatulence	**-**	**-**	**1**	**-**	**-**	**-**	**1**
Diarrhoea	**-**	**-**	**2**	**-**	**-**	**-**	**2**
Myalgia	**-**	**-**	**2**	**-**	**-**	**-**	**2**
Total			**10**				**10**

- Table 15 shows causality assessment of individual ADR in test group.
- All ADRs were categorized as possible under WHO causality assessment scale.

TABLE 16: SEVERITY ASSESSMENT OF ADR

SEVERITY	CONTROL GROUP	TEST GROUP
MILD	**13**	**10**
MODERATE	-	-
SEVERE	-	-

- Table 16 shows severity assessment of Adverse Drug Reactions.
- Severity assessment was done using Modified Hartwig and Siegel scale.
- All the Adverse Drug Reactions in control and study group were mild.

DISCUSSION

DISCUSSION

Coronary heart disease is the most common cause of death worldwide. Abnormalities in lipoprotein metabolism are a major predisposing factor to atherosclerosis, increasing risk for CHD (Coronary heart disease).

Hyperlipidemia characterized by increased levels of total cholesterol, LDL-C and triglycerides, is a major modifiable risk factor in primary and secondary prevention of Coronary Heart Disease. Oxidative stress induced by reactive oxygen species (ROS) is also considered to play an important role in the pathogenesis of hyperlipidemia.

Statins are the most frequently used drugs in the treatment of hyperlipidemia. While statins are highly effective in controlling cholesterol levels, side effects including muscle pain, muscle weakness, and neuropathy are experienced by some patients.

Lycopene is an antioxidant that suppresses cholesterol synthesis and prevents development of atherosclerosis.

Therefore this study was taken up to assess the efficacy of Lycopene in combination with Atorvastatin in patients with Hyperlipidemia.

The patients were screened by history, clinical examination and laboratory investigation.

100 patients who fulfilled the eligibility criteria were enrolled and randomized into two groups of 50 patients in each group. One group received Atorvastatin and the other group received Atorvastatin plus Lycopene for 8 weeks duration.

Hypocholesterolemic effect was assessed by fasting lipid profile and the results were analysed statistically.

The age and sex distribution did not show any statistically significant difference between the study groups. This shows that all the patients belonged to the same population.

Atorvastatin reduces the LDL levels and this was observed in both the groups at the end of the study. But patients receiving Lycopene as add on therapy had a statistically significant reduction in LDL levels ($p < 0.01$). The reduction is about 18% in control group compared to 26% in test group.

At the end of 8 weeks, the total cholesterol levels showed a significant reduction in patients receiving Atorvastatin alone (12.7%) and also in patients with addition of Lycopene to Atorvastatin (17.7%) ($p < 0.001$). On comparing both groups, there was a statistically significant reduction in Total cholesterol levels in patients with Lycopene as add on therapy ($p < 0.001$) in comparison to those receiving Atorvastatin alone

This shows that addition of Lycopene contributes to the reduction of both LDL and Total cholesterol levels. This was similar to the study conducted by Fuhrman et al, where reduction in plasma LDL-C level (14%) was observed.

In Visioli et al study, three weeks supplementation of tomato products showed a significant lycopene concentration in their blood and also reduced oxidizability of LDL suggesting an important role for tomato products in the prevention of lipid peroxidation.

The HDL, VLDL, TGs level did not show any significant difference between the groups. This shows that the addition of Lycopene did not affect these parameters.

The hematological parameters like hemoglobin, total count, differential count, ESR and platelets did not show any significant difference in both study groups at the end of 8 weeks.

There was no significant difference in biochemical parameters like blood sugar, urea, serum creatinine, SGOT, SGPT in both the groups at the end of the study period.

This shows that addition of Lycopene did not affect the hematological and biochemical parameters.

The number of adverse events observed was less in patients receiving Lycopene as add on therapy compared to patients receiving Atorvastatin alone. All the Adverse Drug Reactions were categorized as possible under WHO causality assessment scale. According to the Modified Hartwig and Siegel severity assessment scale all the reactions reported was mild. This shows that Lycopene did not increase the occurrence of adverse events.

As evidenced by earlier studies, our study has also modestly observed that addition of lycopene to Atorvastatin significantly reduced the total cholesterol and LDL cholesterol levels. Addition of lycopene to atorvastatin has not produced changes in hematological, biochemical parameters and adverse drug effect is also mild and thus resulted in significant improvement in the quality of life.

So whether, lycopene may be considered as an alternative to low dose statins or it can be used in combination with low dose statins without side effects in patients with slightly elevated cholesterol levels or whether it can be used as an antioxidant per se requires study with a longer duration.

CONCLUSION

CONCLUSION

From our study, we can conclude that

1. Lycopene as add on therapy to Atorvastatin is effective in reducing lipid levels.
2. Lycopene is well tolerated.

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APPENDICES

APPENDIX - 1

LIST OF ABBREVIATIONS USED

CVD	-	Cardio Vascular Disease
CHD	-	Coronary Heart Disease
LDL-C	-	Low Density Lipoprotein Cholesterol
VLDL-C	-	Very Low Density Lipoprotein Cholesterol
IDL-C	-	Intermediate Density Lipoprotein Cholesterol
HDL-C	-	High Density Lipoprotein Cholesterol
LPL	-	Lipoprotein Lipase
ACAT	-	Acyl CoA Cholesterol Acyltransferase
SREBP	-	Sterol Regulatory Element Binding Protein
CETP	-	Cholesteryl ester transfer protein
LCAT	-	Lecithin Cholesterol Acyl Tranferase
PPAR	-	Peroxisome Proliferator Activated Receptor
AHA	-	American Heart Association
HMG-CoA	-	3-Hydroxyl-3-methylglutarylcoenzyme A
VCAM-1	-	Vascular cell adhesion molecule-1
ROS	-	Reactive Oxygen Species

APPENDIX – 2

CASE REPORT FORM

A PROSPECTIVE, RANDOMIZED, OPEN LABEL, COMPARATIVE STUDY OF ATORVASTATIN ALONE AND ATORVASTATIN WITH LYCOPENE IN PATIENTS WITH HYPERLIPIDAEMIA ATTENDING TERTIARY CARE HOSPITAL

PATIENT DEMOGRAPHY:

NAME :

AGE/SEX :

PLACE :

OP No :

OCCUPATION :

ADDRESS :

CONTACT NUMBER:

DIAGNOSIS :

S.No	Inclusion criteria	Yes	No	Exclusion criteria	Yes	No
1.	25-60 years			Triglycerides > 250		
2.	Total cholesterol level between 200-250 mg/dl			Total cholesterol: HDL ratio > 4.5.		
3.	HT/DM			H/o allergy or intolerance to lycopene		
4.	Patients willing to give written informed consent			Pregnant and lactating women		
5.				Patient with chronic systemic disease		
SUBJECT: INCLUDED ☐ EXCLUDED ☐						
RANDOMISATION: CONTROL GROUP ☐ STUDY GROUP ☐						
REASON IF EXCLUDED:						
SIGNATURE OF PRINCIPAL INVESTIGATOR:						

Medical history:

VITAL SIGNS:

VISITS	VISIT 1	VISIT 2	VISIT 3	VISIT 4	VISIT 5
Pulse rate					
BP					
Temperature					
General/systemic examination					

INVESTIGATIONS:

Hematology Hb	**0 WEEKS**	**8 WEEKS**
TC		
DC		
ESR		
Platelet		
Biochemistry Blood sugar		
Blood urea		
Serum creatinine		
SGOT		
SGPT		

Lipid profile:	**0 WEEKS**	**4 WEEKS**	**8 WEEKS**
Total cholesterol			
HDL cholesterol			
Triglycerides			
LDL-C=[TC-HDL-VLDL]			
VLDL = TG/5			

ADVERSE EFFECTS:

S.No	**ADVERSE EVENTS**	**START DATE**	**STOP DATE**	**TREATMENT GIVEN**

TRIAL CHECK LIST

Particulars	**Screening & Baseline**	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**
Days		**0**	**2 weeks**	**4 weeks**	**6 weeks**	**8 weeks**
Informed Consent	✓					
Randomization	✓					
Patient Demography	✓					
Physical & Clinical examination	✓	✓	✓	✓	✓	✓
Blood pressure	✓	✓	✓	✓	✓	✓
TC,DC, ESR, Hb%	✓					✓
Lipid profile	✓			✓		✓
SGOT, SGPT	✓					✓
Blood Sugar	✓					✓
Blood urea	✓					
Serum creatinine	✓					
Urine analysis	✓					✓
ECG	✓					
Dispense Study Medication		✓	✓	✓	✓	
Adverse Drug Event Monitoring			✓	✓	✓	✓

APPENDIX - 3

INFORMED CONSENT FORM

Title: **"A Prospective, Randomized, Open label, Comparative study of Atorvastatin alone and Atorvastatin with Lycopene in patients with Hyperlipidaemia Attending Tertiary care Hospital**"

Name of the Participant:

I _____ have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and, exercising my free power of choice, hereby give my consent to be included as a participant in this study.

1. I have read and understood this consent form and the information provided to me.
2. I have had the consent document explained to me.
3. I have been explained about the nature of the study.
4. I have been explained about my rights and responsibilities by the investigator.
5. I am aware of the fact that I can opt out of the study at any time without having to give any reasonand this will not affect my future treatment in this hospital.
6. I hereby give permission to the investigators to release the information obtained from me as resultof participation in this study to the sponsors, regulatory authorities, Govt. agencies, and IEC.Iunderstand that they are publicly presented.
7. I have understand that my identity will be kept confidential if my data are publicly presented
8. I have had my questions answered to my satisfaction.
9. I have decided to be in the research study.

I am aware that if I have any question during this study, I should contact the investigator. By signingthis consent form I attest that the information given in this document has been clearly explained to meand understood by me, I will be given a copy of this consent document.

1.Name and signature / thumb impression of the participant (or legal representative if participantincompetent)

Name _____ Signature_____ Date_____

2.Name and Signature of impartial witness (required for illiterate patients):

Name _____ Signature_____ Date_____

Address and contact number of the impartial witness:

Name and Signature of the investigator or his representative obtaining consent:

Name _____ Signature_____ Date_____

APPENDIX - 4
INFORMATION TO PARTICIPANTS

Title: **"A Prospective, Randomized, Open label, Comparative study of Atorvastatin alone and Atorvastatin with Lycopene in patients with Hyperlipidaemia Attending Tertiary care Hospital**"

Principal Investigator:

Name of Participant:

This study is being conducted in Hypertension OPD at Rajiv Gandhi Govt. General Hospital, Chennai. You are invited to take part in this study. The information in this document is meant to help you decide whether or not to take part. Please feel free to ask if you have any queries or concerns.

The purpose of this study

Hyperlipidemia is a major cause of atherosclerosis and atherosclerosis-induced conditions such as coronary heart disease (CHD), ischemic cerebrovascular disease, and peripheral vascular disease. Atherosclerosis remains the major cause of increase in morbidity or mortality in a majority of middle-aged or older adults and account for about one-third of all deaths of persons in this age range . Lycopene, a most predominant carotenoid in human plasma and a natural pigment synthesized by plants and is one of the most potent and most effective antioxidant in protecting the lipid peroxidation of the liposomal membrane, lipid metabolism, and corresponding development of atherosclerosis.Thus we want to test the efficacy and safety of treatment with Lycopene in reducing lipid levels.

We have obtained permission from the Institutional Ethics Committee.

The study design

All patients in the study will be divided into 2 groups A & B. You will be assigned to either of the groups. Group A will receive standard treatment & Group B will receive standard treatment + Lycopene.

Study Procedures

The study involves evaluation of decrease in lipid levels. The planned scheduled visits involve visits at 2^{nd}, 4^{th}, 6^{th}, 8^{th} week after your initial visit. You will be required to visit the hospital 5 times during the study. At each visit, the study physician will examine you. Blood tests will be carried out thrice during the study (at screening , 4^{th} and at the end of study) and total of about 40 ml blood will be collected. These tests are essential to monitor your condition, and to assess the safety and efficacy of the treatment given to you.

In addition, if you notice any adverse events, you have to report it. You will be required to return unused study medicines when you report for your scheduled visits. This will enable correct assessment of the study results.

Possible benefits to you – Lycopene along with standard treatment will cause reduction in lipid levels.

Possible benefits to other people - The results of the research may provide benefits to the society in terms of advancement of medical knowledge and/or therapeutic benefit to future patients.

Confidentiality of the information obtained from you

You have the right to confidentiality regarding the privacy of your medical information (personal details, results of physical examinations, investigations, and your medical history). By signing this document, you will be allowing the research team investigators, other study personnel, and Institutional Ethics Committee to view your data, if required.The information from this study, if published in scientific journals or presented at scientific meetings, will not reveal your identity.

Your decision to not participate in the study affect you

Your decision not to participate in this research study will not affect your medical care or your relationship with the investigator or the institution. You will be taken care of and you will not loose any benefits to which you are entitled.

Can you decide to stop participating in the study once you start

The participation in this research is purely voluntary and you have the right to withdraw from this study at any time during the course of the study without giving any reasons. However, it is advisable that you talk to the research team prior to stopping the treatment/discontinuing of procedures etc.

The expenditure for the treatment and investigation for this study will not be collected from you.

Signature of Investigator Signature of Participant

Date Date

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI -3

EC RegNo.ECR/270/Inst./TN/2013

Telephone No:044 25305301
Fax : 044 25363970
Date: 16.08.2013

CERTIFICATE OF APPROVAL

To
Dr.S.Suganeshwari,
PG in MD Pharmacology,
Madras Medical College, Chennai-3,

Dear Dr.S.Suganeshwari

The Institutional Ethics committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled "A Prospective, Randomized, Open label, Comparative study of Atorvastatin alone and Atorvastatin with Lycopene in patients with Hyperlipidaemia Attending Tertiary care Hospital" No.02082013.

The following members of Ethics Committee were present in the meeting held on 13.08.2013 conducted at Madras Medical College, Chennai -3.

1. Dr.G.SivaKumar, MS FICS FAIS --- Chairperson
2. Prof. R. Nandhini MD -- Member Secretary
   Director, Instt. of Pharmacology ,MMC, Ch-3
3. Prof. Shyamraj MD -- Member
   Director i/c , Instt. of Biochemistry , MMC, Ch-3
4. Prof. P. Karkuzhali. MD -- Member
   Prof., Instt. of Pathology, MMC, Ch-3
5. Prof. Kalai Selvi -- Member
   Prof of Pharmacology, MMC, Ch-3
6. Prof. Siva Subramanian, -- Member
   Director, Instt. of Internal Medicine, MMC, Ch-3
7. Thiru. S. Govindsamy. BABL -- Lawyer
8. Tmt. Arnold Saulina MA MSW -- Social Scientist

We approve the proposal to be conducted in its presented form.

Sd/ Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

R Nandhini 6/9/13
Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003